THE SYSTEMATICS AND ZOOGEOGRAPHY OF THE FRESHWATER CRAYFISH GENUS *Engaeus* ERICHSON (DECAPODA; PARASTACIDAE)

by

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This thesis contains no material which has been accepted for the award of any other degree or diploma in any university, and to the best of my knowledge and belief, contains no copy or paraphrase of material previously published or written by another person, except when due reference is made in the text.

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ABSTRACT

The taxonomy of the freshwater crayfish genus *Engaeus* has been investigated using the techniques of allozyme electrophoresis, multivariate morphometrics and classical taxonomy. The use of these three techniques allows an investigation of both genetic and morphological variation in the genus. It is proposed to synonymize 4 of the previously described species and to erect 14 new species, taking the total number of species in the genus to 34. Based on morphological characters, a partial key to the genera of the Parastacidae and a key to the species in the genus *Engaeus* is given. The description of each species includes a diagnosis, a description of an adult male and an adult female, a discussion of the morphological variation and comments on aspects of the species' life history.

The genealogical relationships of closely related species have been determined from the distance data presented in the electrophoresis section. These relationships are supported with an analysis of ancestral and derived morphological character states, to result in a dendrogram depicting the phylogenetic relationships of the species in *Engaeus* and its allied genera. This dendrogram is compared to previous phylogenies proposed for the parastacids.

In general terms the distribution of *Engaeus* can be described as conforming to the 'Bassian biogeographical region' in south-eastern Australia. Here it has been found to exhibit a high degree of regional endemism; for instance of the 34 species only 2 species occur in both Victoria and Tasmania. In Victoria, 20 endemic species have been recorded. A trend of high diversity and high endemism of the crayfish fauna has been found in highland regions, whilst low levels of both diversity and endemism are recorded for lowland regions. This is attributed to an increase in the number of available habitats in topographically diverse areas of the State. The situation in Tasmania, where 12 species are endemic, is somewhat more difficult to interpret. It seems that the western portion of the island, with its more predictable climatic conditions, can be characterized by broad distributional ranges of a few species, whilst the heterogeneous north-east of the island exhibits many species with reduced, often restricted, geographical ranges.

Four modes of speciation are proposed. The fluctuating sea-levels in the Bass Strait region which accompanied successive glacial and interglacial periods are suggested as the major mechanism in the establishment of geographical barriers between populations.

An absence in the literature of similar studies (on diverse monophyletic groups in the same area) prevents the examination of zoogeographical concordance. Further research is proposed to test the ideas presented in this thesis.

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VOLUME 1

SYSTEMATICS AND ZOOGEOGRAPHY

VOLUME II

MORPHOLOGY AND TAXONOMY

Section 1.1 INTRODUCTION

According to Bowman and Abele (1982), all freshwater crayfish are contained in the infraorder Astacidea Latreille 1803.[1] Within this infraorder the freshwater crayfish are held in two superfamilies, the Astacoidea Latreille 1803 and the Parastacoidea Huxley 1878 (Hobbs, 1974).

Members of the Parastacoidea (all of which are included in the family Parastacidae Huxley 1878) can be distinguished from remaining members of the infraorder on the basis of secondary sexual characteristics (Hobbs, 1974; McLaughlin, 1980). Fourteen genera are presently found in the family. *Astacoides* is recorded from Madagascar (Holthuis, 1964), *Parastacus* and *Samastacus* occur in South America (Riek, 1971) and *Paranephrops* is found in New Zealand (Archey, 1915; Hopkins, 1970). *Astacopsis*, *Cherax*, *Engaeus*, *Engaewa*, *Euastacus*, *Euastacoides*, *Geocharax*, *Gramastacus*, *Parastacoides*, and *Tenuibranchiurus* all occur in Australia (Riek, 1969, 1972; Hobbs, 1974), whilst the genus *Cherax* also occurs in New Guinea (Holthuis, 1949) and on nearby islands (Roux, 1914, 1919; Clark, 1936a).[2]

The fact that all parastacids are found in the Southern Hemisphere was noted by Huxley (1878), Ortmann (1902) and Smith (1912) and has led authors to propose the possibility of a Gondwanaland origin for the family (Bishop, 1967; Williams, 1981) although the absence of parastacids in India and South Africa warrants some explanation if this hypothesis is to be accepted. Riek (1972) presented a phylogeny of the Parastacidae, claiming that the group was monophyletic (contrary to the opinions of Riek, 1959) and that it had its origins in south-eastern Australia, where the family currently enjoys its greatest diversity.

Indeed, it is exclusively in this region of Australia that the genus *Engaeus* can be found (Bishop, 1967; Riek, 1959, 1969; Knott, 1975; Williams, 1981). Until the commencement of this study, 24 species had been described in *Engaeus* (see Riek, 1969 and Suter, 1977a), and preliminary investigations, examining the amount of morphological variation in the genus (see Knott, 1975), showed that this could be an underestimate of the true number of species. This was considered as quite remarkable given the relatively small geographical area in which *Engaeus* occurs. The present project, to investigate the systematic diversity of the genus *Engaeus*, was born with this paradox in mind.

[1]McLaughlin (1980) elevated the family of Austroastacidae Clark 1936 (see Section 1.2) to infraorder rank, apparently on the basis of information to be presented by Bowman and Abele. Since this infraorder was not designated by the latter authors, this infraorder will be disregarded in this thesis.

[2]Morgan (1983) synonymized *Euastacoides* with *Euastacus* and erected a new genus to hold two species from northern Queensland. Since his work has not been published yet his terminology has not been included here.

Species in the genus *Engaeus* are characterized by their ability to burrow, often to considerable depths (Clark, 1936a, 1936b; Riek, 1969), and as a consequence they are relatively cryptic, only rarely being seen above the surface of the ground or in standing water. Ecological studies of these species have been rare until relatively recently (see Clark, 1936b; Suter, 1977a, 1977b; Suter and Richardson, 1977; Richardson and Swain, 1980; Horwitz et al., 1985a; Horwitz et al., 1985b; Horwitz and Richardson, 1986). Precise distributional data for the species are also rare in the literature, with the exception of the work of Suter (1977a) who presented the distributional range of *E. cisternarius*. Physiological studies of *Engaeus* have been limited to an examination of temperature tolerance (Suter, 1975) and to the amount of calcium in the exoskeleton (Mills et al., 1976).

Taxonomic investigations of *Engaeus* have so far been conducted by Smith and Schuster (1913), Clark (1936a, 1939, 1941), Kane (1964), Riek (1951, 1969) and Suter (1977a). However, difficulty in the use of the most recent taxonomic key for this genus (Riek, 1969), coupled with inadequate descriptions of species and the perceived underestimate of documented variation for the genus, have warranted a taxonomic revision.

The present study revises the taxonomy of *Engaeus*, and includes general hypotheses on the phylogeny and zoogeography of the species in the genus.

Section 1.2 TAXONOMIC HISTORY OF *ENGAEUS*

The first published record of *Engaeus* is given by Erichson (1846) for two Tasmanian species in a subgenus of *Astacus*; the subgenus was described with the distinguishing feature of having the outer antennae lying under the inner antennae (antennules), and having gills on the last pair of walking legs. The species *fossor* was described from 6 specimens and was characterized by marginated chelae (propodus) with a double row of 'teeth' along the upper edge, and a marginated (toothed) lower edge. Some proportions of the largest specimen were measured. The species *cunicularius*, described from one specimen, was distinguished from this by a completely smooth outer edge and a feebly toothed inner (upper) edge. Only the locality of Tasmania was given for the species.

Dana (1852) reiterated the general characteristics of *Engaeus* as given by Erichson (1846).

Von Martens (1868) reexamined the material described by Erichson (1846), gave a length for the specimen of *cunicularius* (4.1 cm), said that they live in holes in the ground and provided catalogue numbers for the specimens in the Berlin Zoological Museum (the specimens still have these numbers).

Huxley (1878) discussed the gills of freshwater crayfish, and suggested that the gills of *Engaeus* were the same as those of *Astacopsis* and *Chaeraps* (*Cherax*). The nature and origin of the *Engaeus* material used by Huxley is unknown (see discussion by Kane, 1964).

Haswell (1882) listed the two species in the genus *Engaeus* and suggested that a specimen from Gippsland in the Sydney Museum may belong to the species *fossor*, however the tubercles on the propodus appeared to be different.

Thomson (1892) indicated that Erichson's characters, which were used to separate *fossor* from *cunicularius*, were insufficient. He ascribed a specimen from Zeehan, on the west coast of Tasmania, to the species *cunicularius*.

Ortmann (1902) listed the two species of *Engaeus* (namely *fossor* and *cunicularius*) in an investigation of the distribution of freshwater decapods.

Similarly, Faxon (1898, 1914) catalogued *fossor* and *cunicularius*, and placed inverted commas around the name of *Engaeus*.

Smith (1909) said that the 'land-crayfish' or 'land-crab' occurs

> "...all over the northern and western parts of Tasmania, from sea-level to the tops of the mountains at 4000 ft, on marshy plains or in damp situations in the 'myrtle' forests....of the west coast."

He ascribed the animals to *Engaeus cunicularis* (a mispelling of *cunicularius*). Later, in a paper on the freshwater crayfishes of Austalia, Smith (1912) suggested that *Engaeus* was more closely related to *Chaeraps* and *Parachaeraps* (*Cherax*) than it was to *Astacopsis*, and gave its distribution as Victoria, Gippsland and Tasmania. He recognised a unique morphology in *Engaeus*, with a dorsoventral compression of the body (said to be correlated with a large gastric mill), a total absence of spines on the body, an abdomen which was much reduced in size and variation in the expression of the posterior pleurobranch and the exopodite of the third

maxilliped. In addition, he suggested that *Engaeus* could be split into several subgenera; however this suggestion was not followed in a subsequent taxonomic analysis of the genus (Smith and Schuster, 1913). These authors reiterated the striking morphology of these crayfish and proposed that *Engaeus* was more closely related to *Astacopsis* than it was to other genera. They gave reasonably thorough descriptions of five new species for the genus, namely *affinis*, *phyllocercus*, *victoriensis*, *fultoni* and *hemicirratulus*, all of them from Victoria, as well as re-describing *fossor* and *cunicularius* (where the latter species was recorded from both Victoria and Tasmania, and local varieties of both species were discussed).

The number of described species of freshwater crayfish in Australia blossomed with the publications of Ellen Clark between 1936 and 1942. In her major paper (Clark, 1936a) a new family was erected, the Austroastacidae, to accommodate the new genus *Austroastacus* for *hemicirratulus* and a new species *cymus*. The family was apparently distinguished from the remaining species in the Parastacidae by an absence of the posterior pleurobranch, by a small or absent outer antennule and an absence of the transverse suture of the uropod. In addition, she erected four new parastacid genera, including *Geocharax* and *Pseudengaeus*. In *Geocharax*, two new species were placed, including *lyelli*; the genus was considered to be unique by the shape of the sternal keel between the posterior pair of lateral processes and other characters. Two new species were included in *Pseudengaeus*, namely *strictifrons* and *sternalis*. *Pseudengaeus* also exhibited a unique sternal keel. In *Engaeus*, Clark distinguished *villosus* from *affinis*, *tuberculatus* from *victoriensis*, and *sericatus* and *quadrimanus* from *cunicularius*, and retained *phyllocercus*. *E. fultoni* was considered to be indistinct from *E. fossor* and was consequently synonymized. *E. cunicularius* was restricted to a description of material from the Launceston region of Tasmania.

Clark (1939) examined the Tasmanian Parastacidae and described two new species of *Engaeus*, namely *leptorhynchus* and *ignotus*, the former from the north-east of the State, and the latter, likened to *fossor*, from the north-west corner of the State.

In Clark (1941), two new additions were made to *Geocharax*, including the species *laevis* from Bunyip, Victoria; *E. fultoni* was redescribed and two new species of *Engaeus* were described, namely *marmoratus* and *orientalis*, both from Eastern Victoria.

Riek (1951) described a new species of *Engaeus* from the Australian Capital Territory, *parvulus*, and this represented a significant increase in the known range of the genus. Included in this paper was the description of a new genus of parastacid, *Tenuibranchiurus*, from southern Queensland, which was supposed to have similarities to *Engaeus*.

Guiler (1952), in a list of the Tasmanian Crustacea, listed four species of *Engaeus* (*fossor*, *cunicularius*, *leptorhynchus* and *ignotus*).

Kane (1964) undertook an evaluation of the taxonomy of the freshwater crayfish in Australia, using mainly collections in the Museum of Victoria and his own material ('Kane Collection', now lodged with the Museum of Victoria). His conclusions, with respect to *Engaeus*, were that the family Austroastacidae should lapse, that the genus *Pseudengaeus* was

erected on 'flimsy grounds' and its generic status was not warranted and that the genus *Geocharax* consisted of two groups which were '...at least subgenerically distinct...'(where *laevis* and *lyelli* formed one group). In addition he suggested that *villosus* be synonymized with *affinis*, and that two new species be described from the region east of Melbourne. Kane's work was not published.

Riek (1967) erected a new genus (*Engaewa*) to hold three new species of freshwater crayfish from the south-west of Western Australia. Individuals of this genus were supposed to be morphologically similar to *Engaeus* (see Bishop, 1967).

In Riek (1969) a comprehensive review of the freshwater crayfish of Australia was undertaken, and with respect to *Engaeus*, many of the points raised by Kane (1964) appear to have been heeded. The genera *Pseudengaeus* and *Austroastacus* were synonymized with *Engaeus* (and by implication the family Austroastacidae lapsed) and this was based on the rationale that there was a gradation of structure between all of the species in the three genera. Two species, previously described in *Geocharax* were included in *Engaeus* (*lyelli* and *laevis*) although no explanation was given for this shift. Two species of *Engaeus*, namely *ignotus* and *villosus* were synonymized (to *fossor* and *affinis* respectively). Four new species of *Engaeus* were described, namely *urostrictus*, *connectus*, *australis* and *jumbunna*, all of them from Victoria, east and south-east of Melbourne. Riek produced a key to *Engaeus* and gave diagnoses of new species, but failed to provide diagnoses for the remaining 19 species of *Engaeus*; this frequently rendered use of his taxonomy impossible. For previously described species, no indication was given of exactly which type specimens were examined; where syntypic series were involved no lectotypic or paralectotypic specimens were designated. Generalized distribution maps for each species were figured. Some additions and corrections to the above paper were circulated by the author after its publication.

A phylogeny of the parastacids was provided by Riek (1972) and a description of two new species in a new genus, *Gramastacus*, accompanied his discussion. In the phylogeny, which only dealt with generic relationships, *Engaeus* was depicted as being most closely related to *Parastacus*, and these two genera were more closely related to *Engaewa* and *Tenuibranchiurus* than they were to other genera. *Geocharax* and *Gramastacus* were depicted as being closely related to each other, and more closely related to *Cherax* than to *Engaeus*.

Hobbs (1974) provided a summary of the taxonomic history to that date, listing the family, the 14 genera, and the number of species in each genus in the superfamily Parastacoidea. 23 species were recognised in *Engaeus*.

Suter (1977a) described a new species, *E. cisternarius* from the north-west of Tasmania, demonstrating that it was clearly different to *E. fossor* (with which it had been confused by Smith and Schuster, 1913). Suter (1977b) and Suter and Richardson (1977) made valuable contributions to the knowledge of life history and ecology of *Engaeus*.

Further information on the taxonomic history of each species can be found in the Synonymy and Remarks sections of each Species Description (see Chapter 6).

Section 1.3 SYSTEMATIC METHODOLOGY

A THEORETICAL APPROACH

A fundamental tenet of the taxonomist is that a species is a hypothesis, in other words, a statement or theory that is falsifiable (see Popper, 1972) just like other theories in science. It is therefore incumbent upon the taxonomist to provide a tool which a subsequent biologist can use to test the theory that an organism conforms to a previously described species. Such tools include the taxonomic key and the diagnoses of species, both of which are presently in common use in biology.

It is equally imperative that a taxonomist provides a clear account of the methodology which has been used to delineate the species. In doing so, subsequent workers can easily retrace the steps used to create the classification, thus providing additional avenues through which they can falsify the hypothesis (or hypotheses).

Thus, a general maxim can be followed, namely that the taxonomist should provide data, methodology and descriptions which will allow the hypotheses (species delineations) to be falsified. It seems that some taxonomists in the past have assumed that their methodology is so universal in nature that they have neglected to include any statements about their overall approach and this ultimately renders their taxonomy difficult to use.

The taxonomic methodology commences with an objective overview of the concepts involved in defining a species, and more importantly, the preliminary assessment of the organisms under investigation. The fundamental concept to the taxonomist is the definition of the species, or in other words the answer to the question 'on what basis are individuals grouped together to form a classificatory unit (a species)?'. This may, for instance, be referred to as the 'biological species concept', or alternatively it may be fashioned to meet the particular requirements of the study.

The preliminary assessment of the organisms under study is often philosophically difficult to grasp, since it is here that the initial decisions are made to determine which particular individuals will be examined to create the classification. Ideally, a taxonomist will include every individual which is encountered, and in doing so eliminate the subjective decisions inherent in subsampling. This ideal situation is infrequently attainable due to such factors as insufficient sample sizes or the time constraints involved in the sheer enormity of the overall task. Thus the taxonomist is forced to use selected individuals which are considered to be representative of the overall range of the differences between units within the group under investigation; these selected individuals have been termed the Operational Taxonomic Units (sensu Sneath and Sokal, 1973; hereafter referred to as OTUs). The subjectivity involved in the selection of the OTUs is most difficult to avoid. However, provided that a clear description of the selective technique accompanies the OTUs, the maxim stated above will be satisfied.

The definition of the species and the method by which both the taxonomic techniques and the OTUs are selected, are a priori decisions in the taxonomic analysis.

The next step in the taxonomic methodology involves the actual analyses of the

OTUs. For each analysis, the null hypothesis of either "all OTUs are the same" or "all OTUs are different", is applied, and the analytical technique is used to either reject or accept the null hypothesis. The analyses of OTUs are therefore filtering processes through which an objective final decision is made on the make-up of the species hypothesis.

Having made the decision as to what groups of individuals constitute a species, in other words having completed the '*alpha* taxonomy', as some biologists describe it, '*beta* taxonomy' can be analysed. Here hypotheses can be proposed on how the species evolved and from whom (mode of speciation and genealogical relationships or phylogeny) and indeed where this took place and how long ago (zoogeography).

THE PRACTICAL APPROACH

In this thesis an attempt has been made to adhere to the above taxonomic guidelines.

The remainder of Chapter 1 deals with the a priori taxonomic decisions. A brief outline of the literature dealing with species concepts is given, culminating in a definition of the species which could be satisfactorily used in this thesis. This is followed by a brief discussion of the taxonomic techniques which are available for parastacid research.

Chapter 2 comprises the selection and analyses of the OTUs; the ultimate aim of this Chapter was to delineate species in the genus *Engaeus*. Chapter 3 incorporates this information into a discussion of the genealogical relationships within the genus, and Chapter 4 introduces a discussion of the species' distributions. Thus Volume I contains the systematics and zoogeography of the freshwater crayfish genus *Engaeus*.

Volume II contains the primary taxonomic information namely the taxonomic keys and the diagnoses of the genera and species. A discussion of the trends in the morphological characters (Chapter 5) serves as an introduction to subsequent taxonomic evaluations. This volume is designed to be self-contained.

Section 1.4 THE SPECIES CONCEPT

There is much literature concerning the definition of the species, and what the term 'species' actually constitutes. It is not the intention of this section to present a synthesis of this material, merely to scan the literature and select from it a definition of 'species' which can be useful in this thesis.

The ultimate aim of this work is to produce a classificatory system for the genus *Engaeus*, and as a unit the term 'species' must obviously fulfil some practical requirements in this regard. However, the simple fact that I intend to examine aspects of the phylogeny and zoogeography of *Engaeus* mean that the definition must have a biological relevance as a classificatory unit (as well as the practical applicability from a human point of view).

The 'biological species concept', stated by Mayr (1963) as being

> '...groups of actually or potentially interbreeding natural populations which are reproductively isolated from other such groups...'

appears to have common acceptance since it may satisfy this biological criterion. The definition does, however, have some theoretical and practical difficulties. Authors such as Paterson (1981) object to the term 'reproductive isolation', since they propose that populations in the same species reproduce according to positive assortative mating and can therefore be defined as having 'specific mate recognition systems' rather than being reproductively isolated from other species.

Whether species are better defined as being 'isolated' or 'recognised' is largely academic from the point of view of most taxonomists since most studies are performed in the absence of any knowledge on reproductive biology, and species must be inferred from perhaps morphological data alone.

These problems can largely be ameliorated by the acceptance of the 'evolutionary species concept' as initially proposed by Simpson (1961):

> '...a single lineage of ancestor-descendant populations which maintains its identity from other such lineages and which has its own evolutionary tendencies and historical fate...'(Wiley, 1978),

since it relies upon phylogenetic information as well as a statement of physical differences, and appears to have as its inherent basis a biological relevance.

Both definitions of the species ('biological species' and 'evolutionary species') have the same genetic consequences. Individuals of a single species will share a high degree of genetic similarity with each other, and individuals of different species will inevitably show genetic differences since separate gene pools are involved. In fact the greater the time since the divergence of the species, the greater the magnitude of this difference. It is the genetic cohesiveness within species which is important in this thesis.

Under these circumstances, to delineate a species one needs a method for the separation of OTUs, and a method which will establish the genetic or genealogical affinities of the OTUs.

With regard to the separation of OTUs, the taxonomic significance of OTUs

which exist together in the same habitat (in sympatry) is relatively clear-cut. For instance, if differences between those OTUs occur, without the presence of intermediary forms, then interbreeding between the OTUs can be ruled out and distinct lineages proposed. However the statuses of geographically separated OTUs which differ only marginally is more difficult to elucidate; in fact the process of delineations of species often involves elements of subjectivity in these cases. For the purposes of this thesis, such delineations of species will be conducted after the collection of as much supplementary information as possible.

In general, therefore, 'species' in this thesis will be delineated after evidence of at least two primary sources has been obtained; each will be characterized by a unique (diagnostic) morphology and by a genetic cohesivenesss.

The classificatory unit of 'subspecies' is usually used under the definition of

> '...an aggregate of local populations of a species, inhabiting a geographic subdivision of the range of the species and differing taxonomically from other populations of the species...'

where "differing taxonomically" is defined as differing by diagnostic characters (Mayr, 1963).

The subspecific category will not be used in this thesis, in an attempt to keep the taxonomic nomenclature of the genus *Engaeus* to a minimum. However the value of identifying and subsequently describing geographical variation is indeed recognized.

The question which now arises is 'how does one collect the information which will satisfy both the practical and theoretical aspects of taxonomy?'

Section 1.5 THE SELECTION OF TAXONOMIC TECHNIQUES

The technique of 'classical taxonomy', or the description of morphology and its variation, is of primary importance since it is the information from which most taxonomic keys and diagnoses of species are constructed. By the inclusion of this technique, this thesis will not deviate from this dogma. By far the majority of studies in parastacid taxonomy have relied entirely on this technique, where morphological characters were provided as evidence from which species could be hypothesized, or alternatively falsified. In addition recent studies have been performed without stating a definition of their species concept and/or without information on reproductive isolation or evolutionary descent of the crayfish (see for instance Swain et al., 1982). These categorizations of individuals can be seen as merely statements of phenetic (morphological) similarity or dissimilarity where the delineations of species were reliant upon the intuition of the taxonomist to determine the systematic value of the morphological variation. The classical approach, therefore, has been criticized for its apparent lack of objectivity (McLaughlin et al., 1982).

Additional techniques should provide further information. For instance ecological and/or distributional data can give concordance with the delineations of species, particularly where species are found in sympatry, and such information has indeed been provided for parastacids (for instance Suter and Richardson, 1977; Richardson and Swain, 1980; Horwitz et al., 1985b) although much work remains to be done.

Numerical techniques are often used in taxonomy, particularly since the advent of computers, in an attempt to introduce an objective assessment of the characters. Analyses of morphometrical data form the bulk of such studies. The advantage of numerical techniques is found in their capacity to deal with large data sets (large numbers of either individuals, OTUs, characters or all of these) to provide a phenetic classification. Such a classification, however, is difficult to interpret phylogenetically, and it remains incumbent upon the biologist to attach the taxonomic categories by incorporating other information such as that gained from a classical approach. Numerical phenetics is at the mercy of the quality of the characters which the taxonomist uses, and this frequently necessitates a good understanding of both the statistical and biological behaviour of the characters. In their respective numerical analyses of independent parastacid genera, Sumner (1978) and Morgan (1983) incorporated both meristic and ordinal characters, and the thorough study of the latter author in particular, revealed some important information. He illustrated the effects of allometry and the importance of interpreting this effect prior to assigning the category of species to his groups of OTUs.

Due to the anticipated magnitude of the number of individuals and OTUs which are to be analysed, this technique has been incorporated in this thesis.

Karyological studies have frequently been used for both delineations of species and for their phylogenetic relationships, often giving data which can be interpreted without the subjective assessment involved for other techniques. This is due in part to the fact that differences between species can be detected as quantum changes rather than gradual ones, where the distinction between ancestral and derived states can be relatively easy to determine

(Baverstock and Schwaner, 1985). For parastacids, preliminary work has revealed very high diploid counts for *Cherax destructor* (2n = 160-180, A. Sokol, Zoology Department, Monash University, personal communication), indicating that comparative studies might be time-consuming and prone to error (Morgan, 1983) and consequently they will not be examined here.

A wide variety of biochemical techniques have been developed to aid the taxonomic researcher. Amongst the more complex of these techniques are those which accurately reflect the base-sequences of nucleic acids, involving the analysis of either the nucleic acid itself or the enzymes which function to cleave the nucleic acid (cleavage site-specific endonucleases; Ferguson, 1980). Along with the process of amino-acid sequencing of proteins, these techniques have not yet been used to study the taxonomy of freshwater crayfish (but see Titani et al., 1984). It seems that the greatest potential for these techniques is in phylogenetic research rather than in the detection of intra- and interspecific variation. This is due in part to the time involved in the preparation of single samples, which prevents the analyses of large (for instance > 5) sample sizes. Despite the fact that they will not be used in this study, serious consideration should be given to their use in the investigation of phylogenetic relationships of predetermined species groups within the parastacid family.

Similarly, serological techniques as described in Ferguson (1980) and Baverstock and Schwaner (1985) appear to have greater applicability in the detection of supraspecific levels of genetic variation. Clark and Burnett (1942) and Patak and Baldwin (1984) have applied these techniques to parastacids, and the latter study in particular has revealed some important phylogenetic results, notably that the genera *Euastacus* and *Astacopsis* were largely indistinguishable on the basis of their haemocyanins. Despite the fact that this technique shows some potential in the elucidation of interspecific variation, it has not been used in this thesis.

Electrophoresis is the major technique of biochemical systematics (Ferguson, 1980). It is the process by which proteins are separated in an electric field according to their differential mobilities, usually determined by the net charge of the protein. In a comparative sense, differential mobility results from differences in the amino-acid composition of the protein, and since amino-acids are coded for by DNA, these differences are assumed to reflect reasonably accurately mutational changes at the gene level. Early electrophoretic studies, which concentrated mainly on the amount of genetic variability within natural populations, revealed that the amount of intraspecific genetic differentiation between populations was small relative to the amount of interspecific differentiation (Avise et al., 1975), an attribute that rendered the technique particularly useful in the delineations of species. Electrophoresis in systematics is based upon the assumptions that Mendelian inheritance applies to the alleles distinguished by it, and that the enzymes analysed are chosen at random with respect to their genetically determined variation. Amongst the disadvantages of the technique, are that it can only detect differences, not similarities, and that the process largely underestimates the degree of genetic divergence between species (Ferguson, 1980). As a technique it has several

advantages over classical taxonomy, such as the immediate identification of hybrids in a population (thus the possible determination of interbreeding species in sympatry) and the detection of the amount of genetic divergence between species thus implying phylogenetic relationships (Richardson et al., 1986). Both of these advantages are important to this study.

The inclusion of three techniques to delineate species in this thesis, namely classical taxonomy, electrophoretic analysis and morphometric analyses, plus the incorporation of ecological and distributional data where relevant, has allowed a rigorous approach to the examination of intra- and interspecific variation in the genus *Engaeus*.

Section 1.6 PHYLOGENY AND ZOOGEOGRAPHY

The study of animal (or plant) distributions needs to be approached from both a descriptive and an interpretive angle. Initially, the base-line data needs to be established by rigorously delineating species or taxa, and by describing their present-day distributions. Included in this initial work is an examination of any environmental variables which can be related with a species' distribution, and a discussion of areas of endemism. The interpretive phase involves the incorporation of phylogenetic hypotheses, vicariant events that might have contributed to a species' distribution and an assessment of each species' dispersal powers, to culminate in an historical account of the monophyletic taxon in that particular area.

A preliminary examination suggested that the genus *Engaeus* is reasonably well-suited to the study of zoogeography. Specimens of most species appeared to have dispersal powers which could be relatively easily categorized, they could be collected readily from generally accessible areas, and the potential for man to interfere with their 'natural' distributions, through the agency of either artificial introductions or exterminations was considered to be limited (they are an unlikely prospect for aquaculture).

The only major disadvantage with using the species of *Engaeus* as zoogeographical indicators is that fossil evidence is very rare and this poses some conceptual problems in the reconstruction of a phylogeny. Parastacid fossils have been described by Wintle (1887), Stirton et al. (1961), Woods (1964), Gill (1973) and they are of predominantly Pleistocene age (Bishop, 1967; Glaessner, 1969). Due to the fact that most of these fossils are incomplete, being either chelae or gastroliths, little or no phylogenetic information can be gained from them, and indeed no parastacid fossils have been attributed to the genus *Engaeus*.

Recently the science of phylogeny has been revolutionized by two relatively independent developments. Hennig (translated into english in 1966) demonstrated that by recognizing derived character states which were held in common by two or more species, monophyletic lineages could be elucidated (although the value of this technique depends very much on the character states in use). Secondly, information from molecular analyses has been used to infer actual changes at the genetic level, and therefore distance data between species which has been derived from this source has phylogenetic implications (Baverstock et al., 1979).

In this thesis, phylogenetic information derived from both of these sources will be used to construct a phylogeny, and this, in turn, will be included in a zoogeographic study which follows the general outlines as presented above.

Section 2.1 SELECTION OF OTUs

Freshwater crayfish specimens were collected by excavating their burrow systems, using the general procedure described by Richardson and Swain (1978). On rarer occasions they were collected by hand from beneath rocks, logs or other large debris in shallow water bodies or they were found swimming freely amongst submerged vegetation where they could be collected by a fine-meshed scoop net. Most of the specimens were preserved in 75 % alcohol and 5 % gycerol and stored in glass vials. The examination of each specimen was performed with the aid of a binocular microscope.

Individual crayfish specimens conforming morphologically to previously described species were collected from either their type localities, or if this was not possible, from nearby sites. Additional material was provided by a preliminary regional survey to search for morphological variants of these described forms. (A subsequent, more detailed regional survey was conducted to establish the geographical range of each morph).

A summary of the resulting OTUs giving their site number and locality, species name (where relevant), and their unique feature(s) is given in Table 1. OTUs were included if they satisfied at least one of the following criteria:

i) historical importance; previously described species were collected from their type localities (abbreviated 'TL' in Table 1) or from sites close to the type localities ('NTL'). In most cases the described species or its type locality was identifiable from the literature; however, for the species *E. cunicularius*, *E. quadrimanus* and *E. marmoratus*, the published diagnoses and keys of Clark (1936a) and Riek (1969) were insufficient to enable identifications to be made, and OTUs which conformed to this group of 'species' are labelled with an asterisk in Table 1.

ii) sympatry; where OTUs occurred in sympatry (at the same site, 'SS' or in the same creek system, 'PS'); these give information about possible hybridization,

iii) geographical speciality; if OTUs occurred at either isolated geographical localities ('IGL') or extremities of a geographical range, or

iv) morphological uniqueness; where individuals of an OTU exhibited undescribed combinations of morphological attributes. Commonly used characters were the presence or absence of pores (of the sternum) and the tuberculation, granulation and setation of the propodus of the chelae.

OTU	Site	Locality	SPECIES	SELECTION CRITERIA
1	V07	1.9 km east of Narracan, South Gippsland, Victoria.	E. phyllocercus	TL
2	V38B	Strzelecki, north of Korumburra, South Gippsland, Victoria.	E. phyllocercus	SS
3	V47B	Ryton Junction, South Gippsland, Victoria.	?	No pores; rounded rostrum; tuberculate ventral propodus.
4	V04	Turtons Pass, Otway Ranges, Victoria.	E. fultoni	NTL
5	V13G	Headwaters of Petticoat Creek, Otway Ranges, Victoria.	E. fultoni	PS
6	V20A	Near Lavers Hill, Otway Ranges, Victoria.	*	IGL
7	V80	Lilly Pilly Gully, Wilsons Promontory, Victoria.	*	IGL; SS
8	V53B	Buln Buln East, near Warragul, Victoria.	*	NTL (for E. quadrimanus); SS
9	V63Q	Labertouche Creek near Warragul, Victoria.	*	NTL (for E. quadrimanus); SS
10	V08	O'Mahonys Creek near Warragul, Victoria.	*	NTL (for E. quadrimanus)
11	V36A	Near Flinders, Mornington Peninsular, Victoria.	*	Granulations over ventral portion of propodal palm
12	V38A	Strzelecki, north of Korumburra, South Gippsland, Victoria.	*	SS
13	T33E	Pats River, near Whitemark on Flinders Island, Bass Strait.	*	IGL
14	T41	Oxberry Creek near Waterhouse, north-eastern Tasmania.	*	IGL
15	T46	Welcome Swamp, north-western Tasmania.	*	IGL
16	V80	Lilly Pilly Gully, Wilsons Promontory, Victoria.	*	IGL; SS
17	V80	Lilly Pilly Gully, Wilsons Promontory, Victoria.	*	IGL; SS
18	V40	Mirboo North, South Gippsland, Victoria.	*	Reduced setation of propodal palm.
19	V45	Near Gormandale, South Gippsland, Victoria.	*	Reduced setation of propodal palm.
20	V55	Near South Buchan, East Gippsland, Victoria.	*	NTL (for E. marmoratus).
20A	V74	Betka River, Hard-to-Seek Road, near Genoa, East Gippsland.	*	Reduced setation of propodal palm.
21	V10	Werribee River, north-west of Melbourne, Victoria.	E. sericatus	Stout chelae; distribution of setae on propodal palm.
22	V28	Near Enfield, south of Ballarat, Victoria.	E. sericatus	Tubercles on ventral surface of propodus.
23	V11	Near Mt. Moriac, west of Geelong, Victoria.	E. sericatus	Elongate chelae; distribution of setae on propodal palm.
24	V22B	Lake Mumblin, south of Terang, western Victoria.	E. sericatus	Distribution of setae on propodal palm.
25	V13H	Petticoat Creek, Otway Ranges, Victoria.	E. sericatus	PS
26	V21B	Just north of Princetown, near the Otway Ranges, Victoria.	E. sericatus	Distribution of setae on propodal palm.
27	V38C	Strzelecki, north of Korumburra, South Gippsland, Victoria.	E. hemicirratulus	SS
28	V41	Near Childers, South Gippsland, Victoria.	E. hemicirratulus	NTL
29	V47A	Ryton Junction, South Gippsland, Victoria.	E. hemicirratulus	SS
30	V46	Koornalla, south of Traralgon, South Gippsland, Victoria.	E. hemicirratulus	No setae on propodal palm of female.
31	V69	Kongwak, near Jumbunna, South Gippsland, Victoria.	E. jumbunna	TL
32	V42	Ti-Tree Creek near Bunyip, south-east of Melbourne, Victoria.	E. laevis	NTL
33	V53A	Buln Buln East, near Warragul, Victoria.	E. laevis	SS; reduced setae on propodal palm
34	V67	Creek just west of Genoa, East Gippsland, Victoria.	E. laevis	IGL
35	V51	Tributary of Buffalo River at Dandongadale, Victoria.	E. cymus	NTL
36	V56	Tributary of Nicholson River near Stratford, eastern Victoria.	E. cymus	IGL
37	V58	Near Beetoomba, north-east Victoria.	E. cymus	Intermediate locality between E. cymus and E. parvulus.
38	N10	Near Yarrangobilly, Snowy Mountains, New South Wales.	E. parvulus	NTL
38A	C01	Condor Creek, near Canberra, A.C.T.	E. parvulus	TL
39	V09A	Yarra River Plains at Warburton, Victoria.	E. affinis	TL; SS
40	V60	Tiger Creek on Heyfield to Jamieson Road, eastern Victoria.	E. affinis	Distant geographic locality.
41	V09B	Yarra River Plains at Warburton, Victoria.	E. affinis	TL; SS
42	V70	On road to Mt. Donna Buang, near Warburton, Victoria.	E. affinis	NTL; propodal palm non-granulate.
43	V71	Kinglake National Park, north of Melbourne, Victoria.	E. affinis	Large size; non-granulate propodal palm; type 3 habitat.
44	V49	Acheron River near Buxton, north-east of Melbourne, Victoria.	E. affinis	NTL (for E. villosus).
45	V05	Near Olinda, Dandenong Ranges, Victoria.	E. victoriensis	NTL
46	V36B	Near Flinders, Mornington Peninsular, Victoria.	E. victoriensis	SS
47	V03	Sherbrooke Forest, Dandenong Ranges, Victoria.	E. tuberculatus	TL; SS
48	V06	Near Powelltown, east of Melbourne, Victoria.	E. connectus	TL
49	V62	Just north of Walhalla, east of Melbourne, Victoria.	E. connectus	IGL
50	X03	Sherbrooke Forest, Dandenong Ranges, Victoria.	E. urostrictus	NGL; SS
51	T01	Lilydale, north-eastern Tasmania.	?	Pores present; uropodal rami without transverse suture.
52	V66	Lind National Park, East Gippsland, Victoria.	E. orientalis	NTL
53	V63S	Labertouche Creek, near Warragul, Victoria.	E. sternalis	NTL
54	V68	Near Mallacoota, East Gippsland, Victoria.	?	Morphologically similar to E. sternalis; IGL.
55	V24	Near Port MacDonnell, South Australia.	E. strictifrons	Large size; western extremity of geographical range.
56	V25	Glenelg River at Dartmoor, western Victoria.	E. strictifrons	NTL
57	V48	Lilly Pilly Gully, Wilsons Promontory, Victoria.	E. australis	TL; SS
58	V02	Near Gisborne, north of Melbourne, Victoria.	E. lyelli	NTL; small adult size.
59	V34	Near Broadford, north of Melbourne, Victoria.	E. lyelli	NTL; large adult size.
60	V31	Near Halls Gap, Grampian Ranges, Victoria.	E. lyelli	Granulation of ventral propodal palm.
61	V33	Rocklands Reservoir, Grampian Ranges, Victoria.	E. lyelli	Western extremity of geographical range.
62	V50A	Burnt Creek, south of Mansfield, Victoria.	E. lyelli	Eastern extension of geographical range.
71	T48	Rocky Cape National Park, north-western Tasmania.	?	Pores present; ventral propodal palm carinate.
72	T44	Near Elizabeth Town, northern Tasmania.	*	Reduced number of sternal pores; propodal palm granulate.
73	T32	Browns Creek near Port Sorell, northern Tasmania.	*	Reduced sternal pores; propodal palm granulate.
74	T40A	Surveyors Creek north of Scottsdale, north-eastern Tasmania.	?	SS; terminal spination of uropodal rami.
75	T40B	Surveyors Creek north of Scottsdale, north-eastern Tasmania.	*	SS; pores present; propodal palm non-granulate.
76	T43	Headwaters of Rubicon River, Deloraine, northern Tasmania.	*	No pores; propodal palm non-granulate.
77	T26Q	Mt. Strzelecki, Flinders Island, Bass Strait.	?	IGL; antennal flagella very long; no pores.
78	T15A	Tributary of Dip River, north-western Tasmania.	E. fossor	SS
79	T45	Geales Creek near Smithton, north-western Tasmania.	E. fossor	Clear expression of posterior sternal pores.
80	T37	Bird River south of Crotty, western Tasmania.	E. cisternarius	Near southern extremity of geographical range.
81	T15B	Tributary of Dip River, north-western Tasmania.	E. cisternarius	TL; SS
82	T20	Weetah, near Elizabeth Town, northern Tasmania.	?	No pores; otherwise as for E. cisternarius.
83	T04B	Pearly Brook, north of Scottsdale, north-eastern Tasmania.	?	Pores present; setose tubercles over propodus.
84	T05	Bradshaws Creek near Herrick, north-eastern Tasmania.	E. leptorhynchus	NTL

TABLE 1: The proposed OTU code, the code for the sampling site (site number), the locality of the sampling site and the selection criteria for each selected group of individuals in the taxonomic analysis of *Engaeus* (see text for explanation).

Section 2.2 ELECTROPHORETIC ANALYSES OF *ENGAEUS*

Introduction

The technique of electrophoresis of proteins is now in widespread use in taxonomic studies. The advantages, disadvantages and the assumptions implicit in its use, are briefly outlined in Chapter 1.

The overall aim of this part of the thesis is to analyse the allelic frequencies from nuclear genome loci by using isozyme electrophoresis, in order to place OTUs from the parastacid genus *Engaeus* into genetically distinct taxa or species.

Electrophoretic studies encompassing species of the decapod family Parastacidae are almost non-existent in the literature, particularly studies of species boundaries within genera. Austin (1979) commenced an examination of the biochemical systematics of the crayfish genus *Cherax* by studying those species which occurred in the south-west of Western Australia; he included a member of the genus *Engaewa* in his analysis of 19 loci on starch-gel medium. His studies have continued and now include most of the described species of *Cherax* and are near completion.

DELINEATION OF SPECIES

The OTUs were selected following an a priori assessment of their relative uniqueness (see Section 2.1). The null hypothesis is therefore that each OTU represents the same taxon and the technique of electrophoresis is the tool with which evidence will be provided to reject or accept the null hypothesis (Richardson et al., 1986). In the absence of any additional information, the delineation of taxa using electrophoretic evidence can be examined in two sets of circumstances:

i) when OTUs are found in sympatry, and

ii) when OTUs are allopatric.

SYMPATRIC OTUs

If sympatric OTUs exhibit allelic frequencies which together do not deviate significantly from the Hardy Weinberg Equilibrium, then the assumptions of the Hardy Weinberg Equilibrium are considered to be viable and the individuals of the sympatric OTUs are interpreted as coming from an interbreeding population of one species. Under these circumstances the null hypothesis is not rejected (since there is no evidence to suggest that the OTUs in question represent different taxa).

However, if the allelic frequencies of sympatric OTUs do deviate significantly from the Hardy Weinberg Equilibrium, then the assumption that the OTUs come from a panmictic or randomly interbreeding population has been violated and evidence is therefore provided to reject the null hypothesis. For instance, if sympatric OTUs exhibit fixed differences at one or more loci, the absence of heterozygotes at these loci indicates that these OTUs are not interbreeding and thus belong to different taxa. (Fixed allelic differences can be defined as those at a locus where none of the individuals of OTU A possess the allele or alleles found in OTU B, and vice versa (Benzie and de Silva, 1984)).

ALLOPATRIC OTUs

The delineation of geographically separated OTUs into taxa is much more difficult, since the assumption that the OTUs belong to a panmictic population cannot be assured and therefore the Hardy Weinberg Equilibrium cannot be invoked. The process relies heavily upon comparisons with previously performed studies to determine levels of divergence (and is therefore somewhat more subjective than the process for sympatric OTUs). This information can come from two sources.

Firstly, the levels of genetic divergence shown for OTUs in sympatry provide a ready-made comparison between legitimate taxa.

Secondly, the levels of genetic divergence given for identified taxa in the literature can be used. A large amount of information can be gleaned from this source; prior to using such material, however, several points of caution need to be made. Primarily, certain electrophoretic techniques provide better resolution than others, with the consequence that the better techniques identify cryptic alleles and some authors have suggested that caution be applied to comparisons between studies which have been undertaken using different techniques (see for example, Thorpe, 1979).

In addition, numerous measures of genetic distance, similarity or identity have been published and for the most part they are likely to give closely correlated results (see Buth, 1984). Probably the least commonly used measure is the % fixed difference, which is unfortunate because it is very easy to calculate and use for comparative purposes. For the purposes of this work, comparisons between other studies will be made by using Nei's genetic distance D (Nei, 1972). Where possible, values of I in the literature will be converted to D using the formula

$$D = -\log_e I \qquad \text{where I = genetic identity (Nei, 1972).}$$

There is now a considerable amount of data in the literature giving levels of genetic distances both between and within species. The information has often been summarized; for instance, Avise et al. (1975) and Thorpe et al. (1978) gave the normal interspecific range of genetic identities I to be 0.3 to 0.8 (corresponding to D values of between 1.204 and 0.223). Adams et al. (manuscript) suggest that 'as a rough rule of thumb' allopatric taxa showing fixed differences at more than 15% of loci studied electrophoretically are most likely to be different species.

The data for decapod species, in particular freshwater species, are likely to be most useful for comparative information. Hedgecock et al. (1982) summarized most of the information for the Crustacea, citing the lowest level of genetic distance between two congeneric species as being 0.083 (between the marine crabs *Ocypode quadrata* and *O. occidentalis*; from Nelson and Hedgecock, 1980) and giving the mean D for crustacean species as 0.528.

Table 1 shows a summary of the literature which is most relevant to this study, including the ranges of values of D found for freshwater decapods. Overall, the lowest interspecific D value was 0.111, whilst the intraspecific values ranged from 0.003 to 0.462

(although this large value almost certainly represents a species complex).

For allopatric OTUs in this study it was decided to err on the side of conservatism by setting a high limit of 0.300 to delineate taxa, corresponding to approximately 25% fixed differences; all OTUs which displayed distances between themselves and other OTUs of above this value were recognised as distinct species. For the OTUs which displayed genetic distances below this level, the null hypothesis was not rejected; they were examined again in sections on morphology and morphometrics for further evidence (Sections 2.3 and 2.4, this Chapter). For marginal cases, those which, for instance, differed by D values of between 0.2 and 0.3, subgroupings were shown suggesting the possible breakdowns into species groups should further information arise.

Having proceeded with the delineations of species (at least as far as can be gone using the electrophoretic analyses), a discussion of the levels of heterozygosities within OTUs and geographical variation between OTUs were given. In addition, the phylogenetic implications of the electrophoretic results were discussed.

GENUS (& Ref.)	No. of Spp.	No. of Loci.	H_I	RANGES OF D VALUES Intraspecific	Interspecific	MEDIUM.
Cherax (1)	6	15	0.008	0.041 - 0.462	0.22 - 1.079	A
Procambarus (2)	5	19	0.016	----------	0.111 - 0.456	A
Orconectes (3)	3	18-25	0.054	0.003 - 0.060	0.31 - 0.41	A
Cambarus (3)	3	15-19	0.053	0.020	0.63 - 0.70	A
Caridina (4)	6	29	0.055	0.039	0.279 - 1.748	B

TABLE 1: The average heterozygosities (per locus, per species; H_I) and intraspecific and interspecific ranges of genetic distances (**D**; Nei, 1972), for 5 genera of freshwater decapods. Included are the number of species examined, the number of loci examined, the electrophoretic medium used (where A = starch gel and B = acrylamide) and the reference (where 1 = Austin, 1979, unpublished, 2 = Brown, 1980, 3 = Nemeth and Tracey, 1979, and 4 = Benzie and de Silva, 1984).

Materials and Methods

OTUs

Sites were selected according to the criteria described earlier (Section 2.1). The OTU (given as a bold number code), the site number and the locality of the site, and the sample site for each OTU are given in Tables 2 and 3. The location of each site is presented in Figure 1; sites where more than one OTU have been identified and collected (sympatric sites) are represented in Figure 1 by multiple (one or more) OTUs.

The analysis was split into the "Victorian Study" comprising OTUs from Victoria (Table 2), and the "Tasmanian Study" (Table 3). This was done to reduce the number of interrelationships between OTUs, and to facilitate an easier interpretation of the results. Whilst a small amount of interrelating between the two sections was performed, each study remains as a discrete entity. Unless otherwise stated, the materials and methods used for each study are identical.

For both species delineation and phylogenetic reconstruction, it is far more important to include many populations for many loci rather than many individuals (see Nei, 1978; Gorman and Renzi, 1979; Richardson et al., 1986). This rationale has been followed here, where sample sizes for each OTU usually varied between 2 and 7 (sample sizes of 1 came from two sources, namely where individuals from the other Study were employed for cross referencing purposes, and at site V80, where 3 individuals yielded 3 electromorphs, therefore splitting 1 OTU into 3 OTUs each with a sample size of 1. (A supplementary study was undertaken for these 3 OTUs (see Appendix III)).

TISSUE SAMPLES

Each individual crayfish bound for the electrophoretic analysis was stored in a polythene freezer bag with a label and immediately frozen in liquid nitrogen. Frozen specimens were transfered on dry ice to the Evolutionary Biology Unit of the South Australian Museum in Adelaide; here the specimens were stored in a -80°C freezer prior to the analysis.

Specimens were thawed and muscle tissue (from the chela and pereiopods, or abdomen) and hepatopancreatic tissue were dissected out and the remainder of the specimen was preserved in 70% ethanol. The hepatopancreatic sample from each specimen was placed in a plastic vial where an equal volume of lysing solution (100 mls of distilled water containing 10 mgs of NADP and 0.1 ml ß-mecaptoethanol) was added to it; this was homogenized using a Branson Sonifier (Model B-12) and then centrifuged at 10 000 g for 10 minutes at 4° C in a Beckman Microfuge. The supernatant was placed in Micro Haematocrit Capillary Tubes; the tubes were sealed at both ends and stored at -20°C until use. The muscle tissue was treated in an identical fashion except that the tissue was hand-ground and homogenized with the lysing solution (rather than being sonified).

ELECTROPHORESIS

All electrophoresis was performed on cellulose acetate gels (Cellogel, Chemetron, Via Gustavo Modena 24-Milan, Italy). The pre-use preparation, and the equipment and techniques employed to set up and load the Cellogel, to stain, incubate and

OTU	n	Site	Locality
1	3	V07	1.9 km east of Narracan, South Gippsland, Victoria.
2	3	V38B	Strzelecki, north of Korumburra, South Gippsland, Victoria.
3	3	V47B	Ryton Junction, South Gippsland, Victoria.
4	4	V04	Turtons Pass, Otway Ranges, Victoria.
5	3	V13G	Headwaters of Petticoat Creek, Otway Ranges, Victoria.
6	2	V20A	Near Lavers Hill, Otway Ranges, Victoria.
7	1	V80	Lilly Pilly Gully, Wilsons Promontory, Victoria.
8	3	V53B	Buln Buln East, near Warragul, Victoria.
9	3	V63Q	Labertouche Creek near Warragul, Victoria.
10	3	V08	O'Mahonys Creek near Warragul, Victoria.
11	2	V36A	Near Flinders, Mornington Peninsular, Victoria.
12	3	V38A	Strzelecki, north of Korumburra, South Gippsland, Victoria.
13	2	T33E	Pats River, near Whitemark on Flinders Island, Bass Strait.
14	4	T41	Oxberry Creek near Waterhouse, north-eastern Tasmania.
15	4	T46	Welcome Swamp, north-western Tasmania.
16	1	V80	Lilly Pilly Gully, Wilsons Promontory, Victoria.
17	1	V80	Lilly Pilly Gully, Wilsons Promontory, Victoria.
18	3	V40	Mirboo North, South Gippsland, Victoria.
19	3	V45	Near Gormandale, South Gippsland, Victoria.
20	3	V55	Near South Buchan, East Gippsland, Victoria.
21	3	V10	Werribee River, north-west of Melbourne, Victoria.
22	3	V28	Near Enfield, south of Ballarat, Victoria.
23	5	V11	Near Mt. Moriac, west of Geelong, Victoria.
24	2	V22B	Lake Mumblin, south of Terang, western Victoria.
25	2	V13H	Petticoat Creek, Otway Ranges, Victoria.
26	3	V21B	Just north of Princetown, near the Otway Ranges, Victoria.
27	3	V38C	Strzelecki, north of Korumburra, South Gippsland, Victoria.
28	3	V41	Near Childers, South Gippsland, Victoria.
29	3	V47A	Ryton Junction, South Gippsland, Victoria.
30	3	V46	Koornalla, south of Traralgon, South Gippsland, Victoria.
31	4	V69	Kongwak, near Jumbunna, South Gippsland, Victoria.
32	4	V42	Ti-Tree Creek near Bunyip, south-east of Melbourne, Victoria.
33	2	V53A	Buln Buln East, near Warragul, Victoria.
34	2	V67	Creek just west of Genoa, East Gippsland, Victoria.
35	6	V51	Tributary of Buffalo River at Dandongadale, Victoria.
36	2	V56	Tributary of Nicholson River near Stratford, eastern Victoria.
37	3	V58	Near Beetoomba, north-east Victoria.
38	2	N10	Near Yarrangobilly, Snowy Mountains, New South Wales.
39	4	V09A	Yarra River Plains at Warburton, Victoria.
40	2	V60	Tiger Creek on Heyfield to Jamieson Road, eastern Victoria.
41	3	V09B	Yarra River Plains at Warburton, Victoria.
42	2	V70	On road to Mt. Donna Buang, near Warburton, Victoria.
43	5	V71	Kinglake National Park, north of Melbourne, Victoria.
44	3	V49	Acheron River near Buxton, north-east of Melbourne, Victoria.
45	2	V05	Near Olinda, Dandenong Ranges, Victoria.
46	2	V36B	Near Flinders, Mornington Peninsular, Victoria.
47	3	V03	Sherbrooke Forest, Dandenong Ranges, Victoria.
48	2	V06	Near Powelltown, east of Melbourne, Victoria.
49	3	V62	Just north of Walhalla, east of Melbourne, Victoria.
50	3	X03	Sherbrooke Forest, Dandenong Ranges, Victoria.
51	1	T01	Lilydale, north-eastern Tasmania.
52	4	V66	Lind National Park, East Gippsland, Victoria.
53	3	V63S	Labertouche Creek, near Warragul, Victoria.
54	3	V68	Near Mallacoota, East Gippsland, Victoria.
55	2	V24	Near Port MacDonnell, South Australia.
56	3	V25	Glenelg River at Dartmoor, western Victoria.
57	4	V48	Lilly Pilly Gully, Wilsons Promontory, Victoria.
58	2	V02	Near Gisborne, north of Melbourne, Victoria.
59	2	V34	Near Broadford, north of Melbourne, Victoria.
60	2	V31	Near Halls Gap, Grampian Ranges, Victoria.
61	2	V33	Rocklands Reservoir, Grampian Ranges, Victoria.
62	2	V50A	Burnt Creek, south of Mansfield, Victoria.

Table 2: The OTU code, sample size (n), site code and locality of the collection for each OTU in the Victorian study. OTU **51** (from site T01) was taken from the Tasmanian Study and is used here for comparative purposes. (For more specific details of localities refer to PREFACE TO SPECIES DESCRIPTIONS in VOL. II).

OTU	n	Site	Locality
71	5	T48	Rocky Cape National Park, north-western Tasmania.
72	5	T44	Near Elizabeth Town, northern Tasmania.
73	5	T32	Browns Creek near Port Sorell, northern Tasmania.
74	6	T40A	Surveyors Creek north of Scottsdale, north-eastern Tasmania.
75	5	T40B	Surveyors Creek north of Scottsdale, north-eastern Tasmania.
76	5	T43	Headwaters of Rubicon River, Deloraine, northern Tasmania.
77	6	T26Q	Mt. Strzelecki, Flinders Island, Bass Strait.
78	5	T15A	Tributary of Dip River, north-western Tasmania.
79	5	T45	Geales Creek near Smithton, north-western Tasmania.
80	7	T37	Bird River south of Crotty, western Tasmania.
81	5	T15B	Tributary of Dip River, north-western Tasmania.
82	5	T20	Weetah, near Elizabeth Town, northern Tasmania.
51	5	T01	Lilydale, north-eastern Tasmania.
83	5	T04B	Pearly Brook, north of Scottsdale, north-eastern Tasmania.
84	5	T05	Bradshaws Creek near Herrick, north-eastern Tasmania.
14	1	T41	Oxberry Creek near Waterhouse, north-eastern Tasmania.
57	1	V48	Lilly Pilly Gully, Wilsons Promontory, Victoria.
31	1	V69	Kongwak, near Jumbunna, South Gippsland, Victoria.

Table 3: The OTU code, sample size (n), site code and locality of the collection for each OTU in the Tasmanian study. OTUs **14, 31** and **57** (from sites T41, V69 and V48 respectively) were taken from the Victorian Study and are used here for comparative purposes. (For more specific details of localities refer to PREFACE TO SPECIES DESCRIPTIONS in VOL. II).

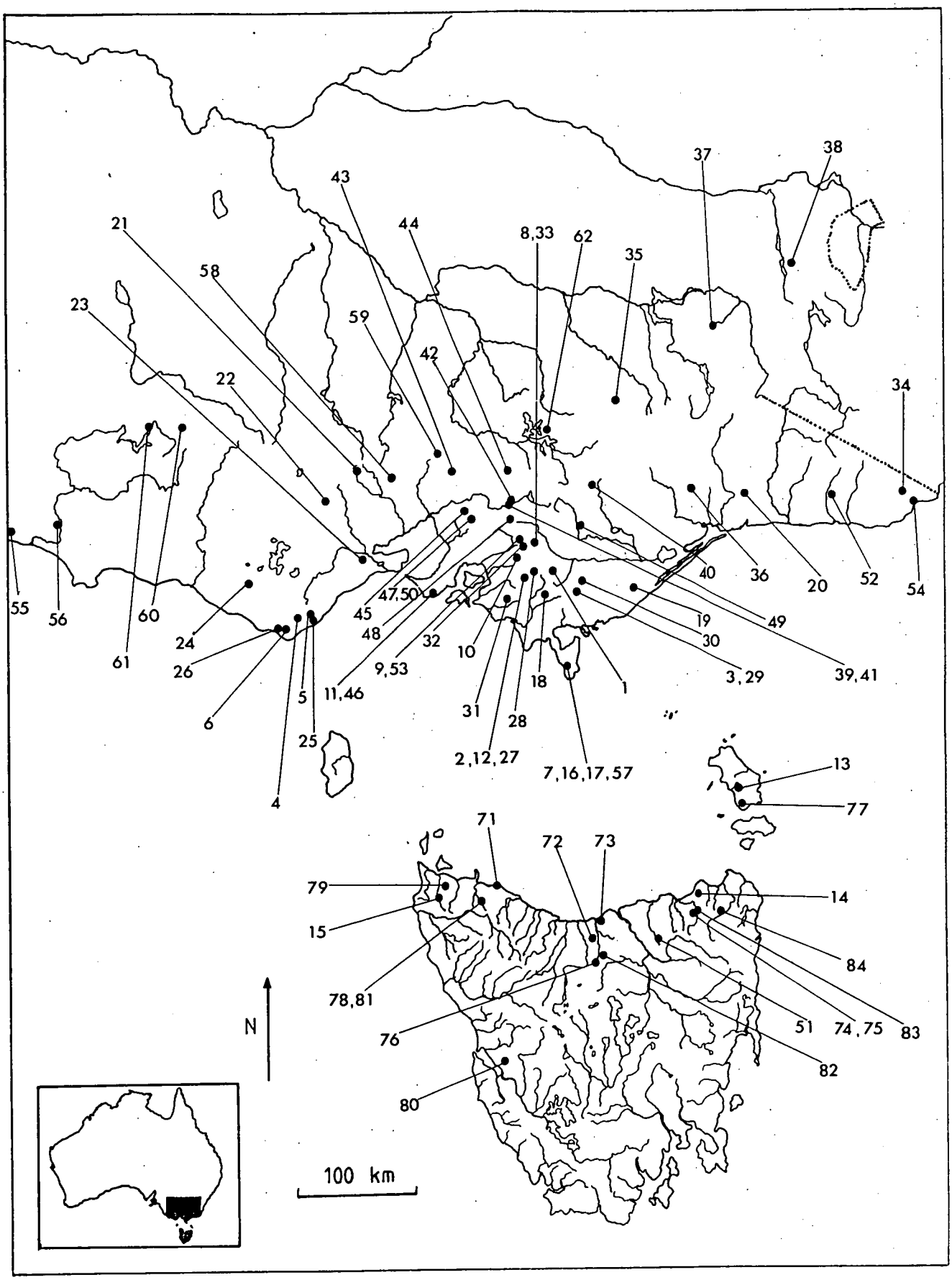

Figure 1: Localities of collecting sites of OTUs for both the Victorian and Tasmanian Studies. See Tables 2 and 3 for OTU codes. Sites of sympatry are represented by more than one OTU.

score the gels and finally to interpret the gels, are described in detail in Richardson et al. (1986).

ENZYMES

A total of 38 enzymes were examined in these studies. Seventeen enzymes were either too weak to score, inconsistent in their expression or were genetically uninterpretable and these were excluded from the data. They included adenosine deaminase and alkaline phosphatase from the hepatopancreas, and adenylate kinase, diaphorase, esterase, guanine deaminase, glutamate dehydrogenase, glyoxalase I, glucose-6-phosphate dehydrogenase, (alpha)glycerophosphate dehydrogenase, hexokinase, malic enzyme, nucleoside phosphorylase, peptidase (two loci), phosphoglycerate mutase, phosphoglucomutase and pyruvate kinase from muscle tissue.

The enzymes encoding for the remaining 21 loci are presented in Table 4 with their abbreviations, enzyme commission (E.C.) number and the electrophoretic buffers in which they were run. The stains used for each enzyme are given in Richardson et al. (1986). Two of the 21 enzymes originated from the hepatopancreatic tissue (ADH and SORDH) and the rest came from the muscle sample. In general the enzymes were run at 200 Volts at 4°C for 100 minutes (all except for FUM which was run for 140 minutes, PhDH which was run for 130 minutes, ALD, PEP-B and 6PGD which were all run for 80 minutes and MPI which was run for 40 minutes).

20 loci were utilized for the Victorian Study (PEP-B was excluded from this analysis) and 20 loci were utilized for the Tasmanian Study (6PGD was excluded from this analysis).

ANALYSIS OF DATA

In the analysis percent fixed differences and Nei's standard genetic distance ('D', Nei, 1972) were used as methods of measuring the extent of genetic divergence between groups. Richardson et al. (1986) outline the pros and cons of the most frequently used methods; they give examples and comparisons to other units of measurement to argue that % fixed differences can be used as an accurate and "practical" measure between groups in a taxonomic study (except in studies where the organisms are extremely polymorphic, but this does not apply here). The practical advantage for % fixed differences is their ease of calculation. Nei's genetic distance has a more classical role in electrophoretic studies and its inclusion in this analysis will facilitate the comparisons with other decapod work. It takes into account not only the fixed differences between OTUs but also the frequencies of alleles at polymorphic loci and it is said be advantageous since it measures a biological property (the mean number of electrophoretically detectable substitutions per locus that have accumulated since the two populations diverged from their common ancestor; Buth, 1984). In the analysis it has been corrected for small sample size (Nei and Roychoudhury, 1974; Nei, 1978).

For each of the measures of genetic divergence, a matrix of the differences between all of the OTUs was compiled from the gene frequency data. From these matrices, numerical methods were used to construct a phylogeny using a phenetic technique and a

ABBR.	COMMON NAME	E.C. NUMBER	BUFFER	Sub-Units
6PGD	6 Phosphogluconate dehydrogenase	1.1.1.44	B	2
ADH	Alcohol dehydrogenase	1.1.1.1	C	2
ALD	Aldolase	4.1.2.13	A	4
ENOL	Enolase	4.2.1.11	A	2
FDPase	Fructose-diphosphatase	3.1.3.11	B	4
FUM	Fumarase	4.2.1.2	B	4
GA3PD	Glyceraldehyde-3-phosphate dehydrogenase	1.2.1.12	B	4
GOT-1	Glutamate-oxaloacetate transaminase	2.6.1.1	B	2
GOT-2	Glutamate-oxaloacetate transaminase	2.6.1.1	B	2
GPI	Glucose-phosphate isomerase	5.3.1.9	A,C	2
GPT	Glutamate-pyruvate transaminase	2.6.1.2	B	2
IDH	Isocitrate dehydrogenase	1.1.1.42	A	2
LDH	Lactate dehydrogenase	1.1.1.27	B	4
MDH-1	Malate dehydrogenase	1.1.1.37	A	2
MDH-2	Malate dehydrogenase	1.1.1.37	A	2
MPI	Mannose-phosphate isomerase	5.3.1.8	B,A	1
PEP-B	Peptidase	3.4.11	A	1
PGK	Phosphoglycerate kinase	2.7.2.3	A,B	1
PhDH	General protein		B	1
SORDH	Sorbitol dehydrogenase	1.1.1.14	C	4
TPI	Triose-phosphate isomerase	5.3.1.1	B	2

Table 4: The abbreviations, common names and E.C. numbers of the enzymes which were stained and scored, with the buffer used for each enzyme (where A = 0.01 M citrate-phosphate, pH 6.4; B = 0.02 M phosphate, pH 7.0; C = 0.05 M Tris-maleate, pH 7.8; see Richardson et al., 1986 for more details), and the sub-unit structure of each enzyme.

phylogenetic technique.

The phenogram was constructed using the unweighted pair group method with arithmetic averages (or UPGMA; Sneath and Sokal, 1973). This method examines the data matrix and forms its first cluster from the most similar OTU pair (which from then on is treated as a single entity); the data matrix is then recalculated and from this the most similar OTU is added to the initial cluster; this process is continued until all OTUs have been clustered (Ferguson, 1980). This technique presents the genetic relationships of the OTUs in a two dimensional form (Richardson et al., 1986). Whilst the interpretation of homology for these clusters is relatively accurate when the differences between OTUs are small, it becomes less accurate as the differences between the OTUs increase to higher levels (for reasons given in Richardson et al., 1986) and so caution must be exercised. In addition, to infer a phylogenetic relationship from such a phenetic technique involves the critical assumptions that the rates of evolution of the lineages (in this case OTUs) are equal and the distances from the origin to each of the OTUs is the same.

The second numerical method, namely the Distance Wagner (Farris, 1972) constructs hypothetical taxonomic units (HTUs) between sample populations (OTUs) to obtain the shortest total patristic length (Farris, 1970) between all OTUs. The Wagner tree method therefore attempts to find the most parsimonious set of phylogenetic relationships between the groups; it is therefore not dependent upon the assumption of homogeneous rates of evolution (Avise and Smith, 1977). The networks employed in this study are unrooted, in other words no evolutionary direction or polarity is assigned (Buth, 1984). Farris (1972) describes three methods for rooting a Wagner network, namely by using outgroups, by assuming that evolutionary rates are equal or by minimizing the variance of evolutionary rates. The use of outgroups assumes that all OTUs in the study are monophyletic or derived from the same stock (see Watrous and Wheeler, 1981), but there was no a priori information available at the commencement of this study which could verify such an assumption. In addition, since the Distance Wagner has been chosen to avoid the assumption of homogeneous rates of evolution and thus provide a valid comparison to the phenetic results, it was not rooted.

Results

ENZYMES

The sub-unit structure of each enzyme is presented in Table 4. No invariant loci were recorded. Heterozygotes were detected for all loci except GA3PD and ADH.

All enzymes were coded for at one locus, except for MDH and GOT which were both coded for at two loci (cytosol loci GOT-1 and MDH-1, and mitochondrial loci GOT-2 and MDH-2).

GENETIC VARIATION - TASMANIAN STUDY

The allele frequencies at each locus are given in Table 5. These data show that a total of 97 alleles were observed at the 20 loci. Within populations (OTUs), an average of only 1.039 alleles per locus was recorded. This is in accordance with the very low figures for the average estimated heterozygosity per locus and proportion of polymorphic loci (H_E and P, Table 6). Both measures of genetic variation given in Table 6 show close a correlation. The overall average estimated heterozygosity per locus per species in the Tasmanian Study (but excluding the OTUs of **57** and **31** from Victoria) is 0.011 (see Discussion).

The genetic difference between OTUs for Nei's D and % fixed differences are presented in matrix form in Table 7; phenetic clusterings of the OTUs using the UPGMA are given in Figure 2. Figure 3 shows the Distance Wagner Trees derived from both dets of data.

GENETIC VARIATION - VICTORIAN STUDY

The allele frequencies at each locus are given in Table 8. These data show that a total of 128 alleles were observed at the 20 loci. Within populations (OTUs) an average of only 1.048 alleles per locus was observed.

As with the Tasmanian Study, a low level of heterozygosity was found. This is possibly best exemplified at the MDH-1 locus where 13 alleles were detected without any heterozygotes. The average estimated heterozygosities (H_E), observed heterozygosities (H_O) per locus and proportion of polymorphic loci (P) are given in Table 9. Linear regressions were performed between each measure of genetic variation given in Table 9 and in each case a significant r^2 value was obtained (for H_E and H_O, $r^2 = 0.7380$, $F_{df=60} = 168.997$, $p < 0.001$; for H_E and P, $r^2 = 0.9430$, $F_{df=60} = 992.219$, $p < 0.001$; for H_O and P, $r^2 = 0.7041$, $F_{df=60} = 142.774$, $p < 0.001$). The overall average heterozygosity per locus per species (calculated without OTU **51** from Tasmania) is 0.020.

The genetic difference matrix for the Victorian Study is given in Table 10. The phenetic clusterings of OTUs are shown in Figure 4 and the Distance Wagner Trees are depicted in Figure 5.

DELINEATION OF SPECIES - TASMANIAN STUDY

Sympatric OTUs

On a considerable number of occasions, fixed allelic differences between OTUs were recorded. This is particularly important when considering the two pairs of sympatric OTUs:

i) **74** & **75** (which show fixed differences at the FDPase, GOT-1, GOT-2, GPI, GPT, IDH,

LOCUS	Allele	71	72	73	74	75	76	77	78	79	80	81	82	51	83	84	14	57	31
ADH	B										100	100	100		100		100		100
	A	100	100	100	100	100	100	100	100	100				100		100		100	
ALD	F																	100	
	E															100			
	D							100			100	100	100						
	C		100	100	100	100	100							100	100		100		
	B																		100
	A	100							100	100									
ENOL	D							100											
	C	100	100	100	100	100	100		100						100	100	100	100	
	B										100	100							
	A									100			100	100					100
FDPase	B		100	100	100		100		100	100	100	100	100				100	100	100
	A	100				100		100						100	100	100			
FUM	D	10																	
	C	90	100	100	100	100	100	100	100	100	100	90	90				100		100
	B															100		100	
	A											10	10	100	100				
GA3PD	C	100									100				100		100		100
	B		100	100	100	100	100	100	100	100		100	100	100		100			
	A																	100	
GOT-1	H																		100
	G				100				100	100					100		100	50	
	F											100							
	E										100		100					50	
	D	100	100	100		100	100												
	C							100											
	B													13					
	A													87		100			
GOT-2	G	100																	
	F														100				
	E		100	100	100				50	40							100	100	100
	D					100	100	100											
	C								50	60				100		100			
	B										100	100							
	A												100						
GPI	H																	100	
	G					100													
	F																		100
	E				100														
	D		100	100			100	100						100		100			
	C														100		100		
	B								100	100	100	100	100						
	A	100																	
GPT	E				100														
	D	100							100	100	100	100				100			
	C		100	100		100	100	100					100	100			100		100
	B														100				
	A																	100	
IDH	C		100	100		90			100	100	100	100	100	100	100	100	100		
	B	100			100		100	100										100	
	A					10													100
LDH	F								100	100									100
	E		100	100	100														
	D														100				
	C	100					100	100			100	100	100	100		100			
	B					100													
	A															100		100	
MDH-1	H										100	90							
	G												100						
	F											10							
	E						100												
	D	100								80								100	
	C																		100
	B		100	100	100			100						100	100	100			
	A					100			100	20							100		
MDH-2	F																		100
	E												100						
	D	100	100	100	100	100	100	100	100	100	100	100		100		20	100		
	C														100				
	B															80			
	A																	100	
MPI	B		100	100	100	100	100												
	A	100						100	100	100	100	100	100	100	100	100	100	100	100
PEP-B	H				100			100	100	100						17			
	G												100						
	F													100				100	100
	E		100	100		100										83			
	D														100				
	C	100					100												
	B											70					100		
	A										100	30							
PGK	F	100					100												
	E		100	100		100										100			
	D																100	100	100
	C				100				100	100									
	B							100			100	100	100		100				
	A													100					
PhDH	B	100	100	100	100	100	100	100	100	100				100		100	100	100	100
	A										100	100	100		100				
SORDH	D			100		100													
	C				100										100				
	B		100				100	100	37	100	100	100	100	100		100	100	100	100
	A	***							63										
TPI	C	100	100	100	100	100	100		100	100	100	100	100		100	100	100	100	100
	B													100					
	A							100											

Table 5: The gene frequencies at 20 loci for the OTUs in the Tasmanian Study. Alleles are listed alphabetically in order of increasing distance from the cathode. Asterisks indicate the failure of all individuals to stain at a particular locus.

OTU	H_E	P	OTU	H_E	P
71	0.009	0.053	**80**	0	0
72	0	0	**81**	0.039	0.150
73	0	0	**82**	0.009	0.050
74	0	0	**51**	0.011	0.050
75	0.009	0.050	**83**	0	0
76	0	0	**84**	0.015	0.100
77	0	0	**14**	0	0
78	0.048	0.100	**57**	0.025	0.050
79	0.040	0.100	**31**	0	0

Table 6: The average estimated heterozygosities per locus per OTU (H_E) and the proportion of polymorphic loci for each OTU (**P**) in the Tasmanian Study.

OTU	71	72	73	74	75	76	77	78	79	80	81	82	51	83	84	14	57	31
71		1008	1008	1008	867	550	872	738	752	1008	1156	1596	1141	1330	947	872	966	1350
72	63		51	431	359	357	799	679	732	1204	1042	1059	687	1386	578	598	1024	1050
73	63	5		431	289	431	916	717	845	1386	1199	1216	792	1386	673	693	1178	1204
74	63	35	35		793	598	916	526	631	1609	1384	1630	1198	1204	1127	799	955	1386
75	58	30	25	55		506	911	777	1008	1630	1400	1417	918	1401	784	805	1579	1580
76	42	30	35	45	40		598	843	894	1204	1042	1059	792	1897	1005	693	1024	1204
77	58	55	60	60	60	45		971	894	1050	907	924	592	1609	854	1050	1361	1386
78	53	45	50	40	55	55	60		108	843	719	979	1035	1359	728	582	945	1041
79	53	50	55	45	60	60	60	5		776	658	782	815	1587	762	711	906	845
80	63	70	75	80	80	70	65	55	55		135	433	1380	1050	1330	693	1466	1050
81	68	65	70	75	75	65	60	50	50	10		418	1159	1165	1132	712	1562	1199
82	79	65	70	80	75	65	60	60	55	35	35		1024	1182	1364	805	1461	924
51	68	50	55	70	60	55	45	60	55	75	65	60		1198	568	910	1354	1198
83	74	75	75	70	75	85	80	75	80	65	65	65	70		1187	799	1717	1609
84	58	40	45	60	50	60	50	40	45	70	65	75	40	70		1154	874	1592
14	58	45	50	55	55	50	65	40	45	50	50	55	60	55	65		830	598
57	63	65	70	60	80	65	75	55	55	75	80	75	75	80	60	55		891
31	74	65	70	75	75	70	75	60	55	65	70	60	70	80	80	45	60	

Table 7: Genetic difference matrix for OTUs used in the Tasmanian Study, showing % fixed differences below the diagonal and Nei's genetic distance 'D' (Nei, 1972) X 1000, above the diagonal.

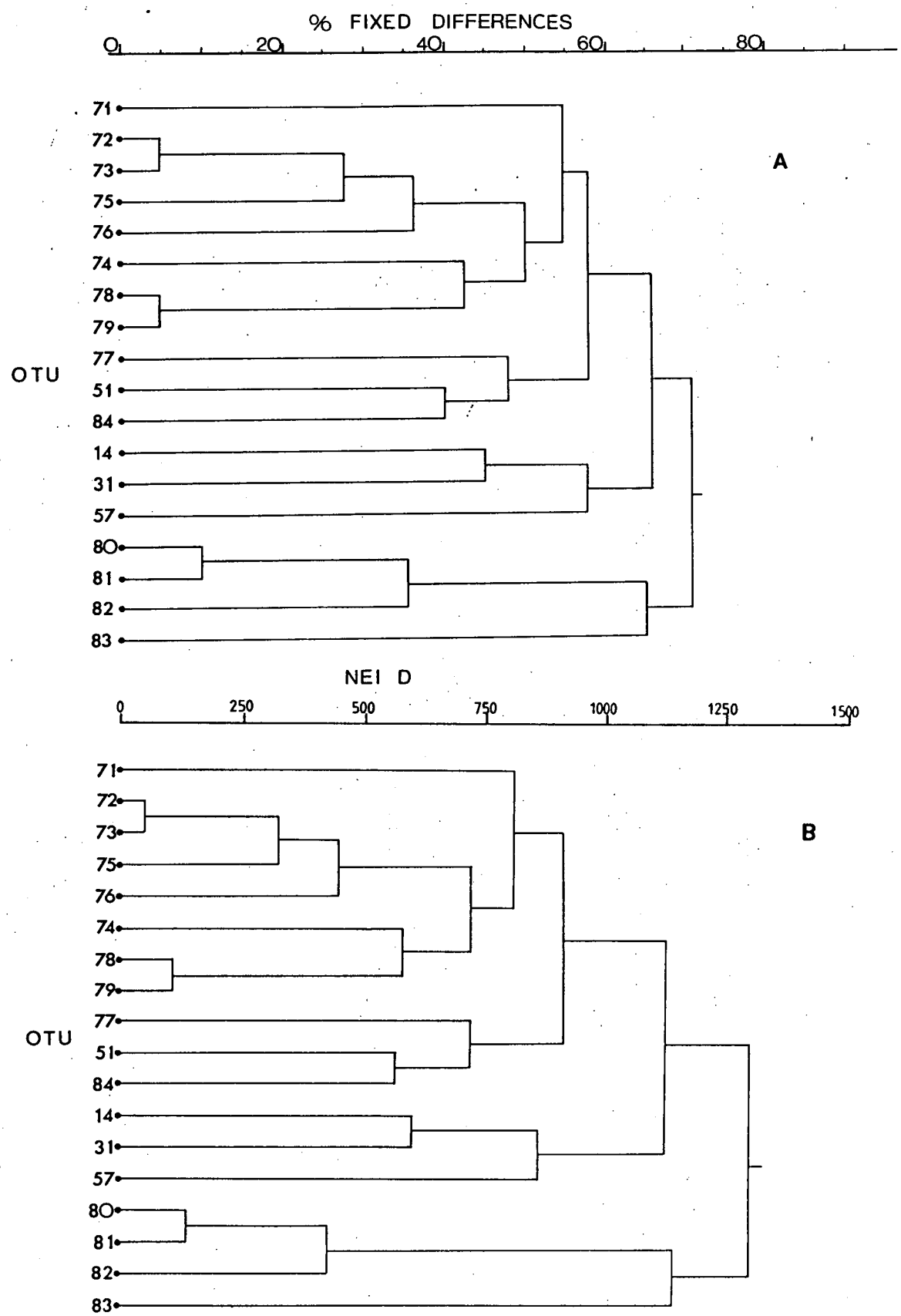

Figure 2: UPGMA clusters derived from the data presented in Table 7 showing the clusters based on % fixed differences (A) and Nei's genetic distance (B).

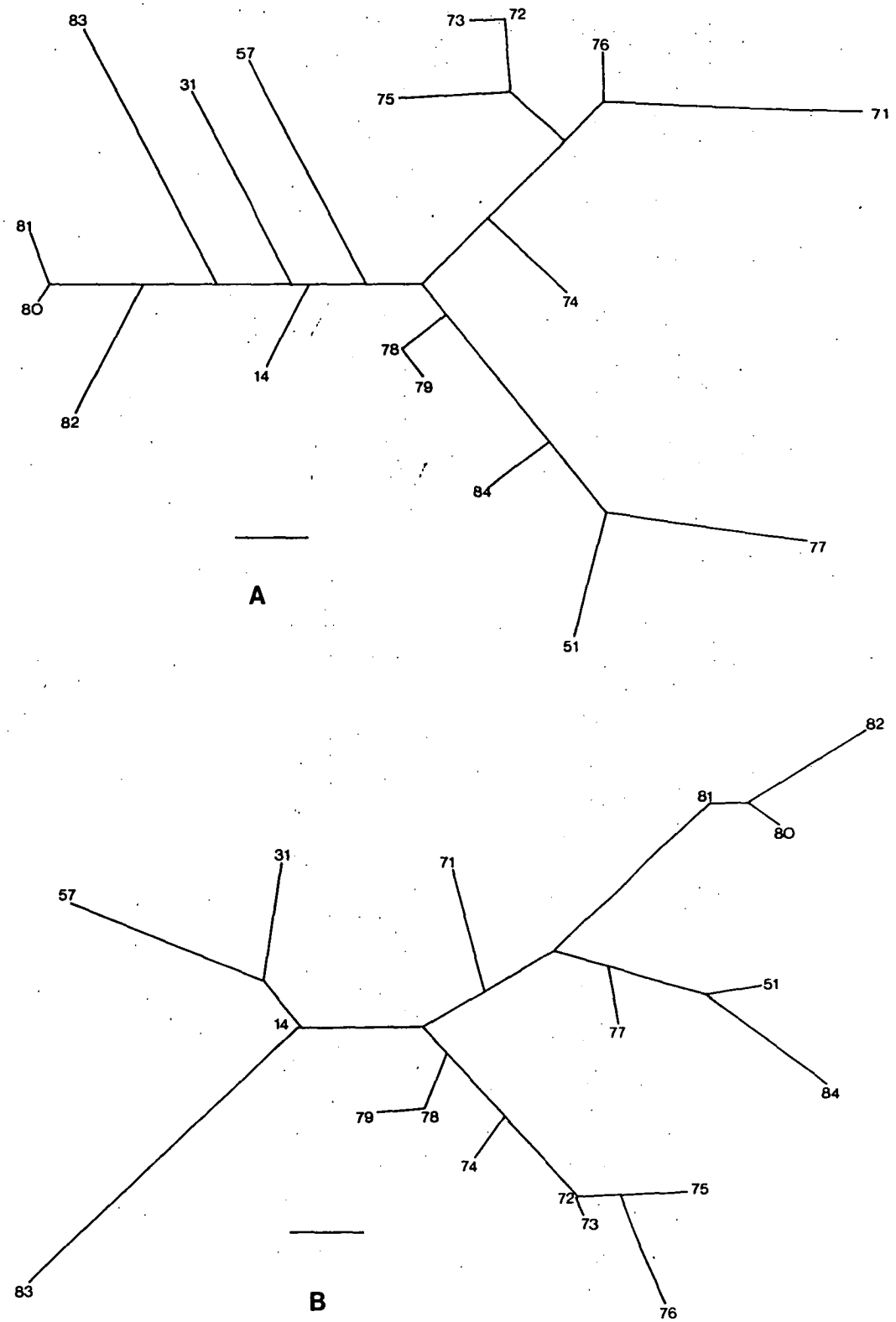

Figure 3: Wagner networks constructed from the data presented in Table 7, based on % fixed differences (A) and Nei's genetic distance (B). Scale bars represent patristic distances of 10 and 0.200 for A and B respectively.

Table 8: The gene frequencies at 20 loci for the OTUs in the Victorian Study. Alleles are listed alphabetically in order of increasing distance from the cathode. Asterisks indicate the failure of all individuals to stain at a particular locus.

LOCUS	Allele	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33
6PGD	F																																	
	E																																	
	D						100	50	67	17	100	100	100																					100	100
	C						100		33	50																								
	B							50	33																									
	A																																	
ADH	F																																	
	D	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
	C																																	
	B																																	
	A																																	
ALD	F																																	
	E				100																													100
	D	100	100																															
	C																																	
	B																																	
ENOL	A	100	100		100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
	B																																	
	A	100																																
EOPass	D																																	
	C																																	
	B	67	67	100	100		100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
	A	17	33																															
FUM	D																																	
	C																																	
	B																																	
	A	100	100																															
GAJPD	D																																	
	C	100	100																															
	B																																	
	A																																	
GOT-1	G																																	
	F																																	
	E	100	100	100																														
	D																																	
	C																																	
	B																																	
	A																																	
GOT-2	G																																	
	F																																	
	E																																	
	D	100	100																															
	C																																	
	B																																	
	A																																	
GPI	I																																	
	H																																	
	G																																	
	F																																	
	E																																	
	D	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
	C																																	
	B																																	
	A																																	
GPT	G																																	
	F	100	100				86																											
	E						12	100	100	100																									
	D	100	100	100	100	100	33	100	100	67	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
	C						88																											
	B						67																											
	A																																	
IDH	F																																	
	E																																	
	D	100	100																															
	C																																	
	B																																	
	A																																	
LDH	F																																	
	E	100	100	100	38	33	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
	D																																	
	C				62	67																												
	B																																	
	A																																	
MDH-1	H																																	
	L																																	
	K																																	
	J																																	
	I																																	
	H																																	
	G																																	
	F																																	
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MDH-2	K																																	
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MPI	A																																	
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PGK	A	100	100	100																														
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	G	100																																
PGM	D	100	100		100	100	100	100	100	100	33		100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
	C																																	
	B																																	
	A				100	50					50	17	100																						
SORDH	D	100	100	100	62	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
	C																																	
	B																																	
	A				25	50																												
TPI	H																																	
	G																																	
	F	100	100	100	73	50	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
	E																																	
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	A				25	50																												

LOCUS	Allele	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62
6PGD	F																													
	E																													
	D							100	100	100																				
	C																													
	B																													
	A																													
ADH	F																													
	D	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
	C																													
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	A																													
ALD	F																													
	E																													
	D	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
	C																													
	B																													
	A																													
ENOL	C	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
	B																													
	A																													
EOPass	D																													
	C																													
	B	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
	A																													
FUM	D																													
	C																													
	B	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
	A																													
GAJPD	D																													
	C																													
	B	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
	A																													
GOT-1	G																													
	F																													
	E																													
	D																													
	C																													
	B																													
	A																													
GOT-2	G																													
	F																													
	E																													
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	C																													
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GPI	I																													
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	E																													
	D	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
	C																													
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	A																													
GPT	G																													
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	E																													
	D	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
	C																													
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IDH	F																													
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	D	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
	C																													
	B																													
	A																													
LDH	F																													
	E	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
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MDH-1	H																													
	L																													
	K																													
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MDH-2	K																													
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MPI	A																													
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PGK	A																													
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	G																													
PGM	D	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
	C																													
	B																													
	A																													
SORDH	D	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
	C																													
	B																													
	A																													
TPI	H																													
	G																													
	F	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
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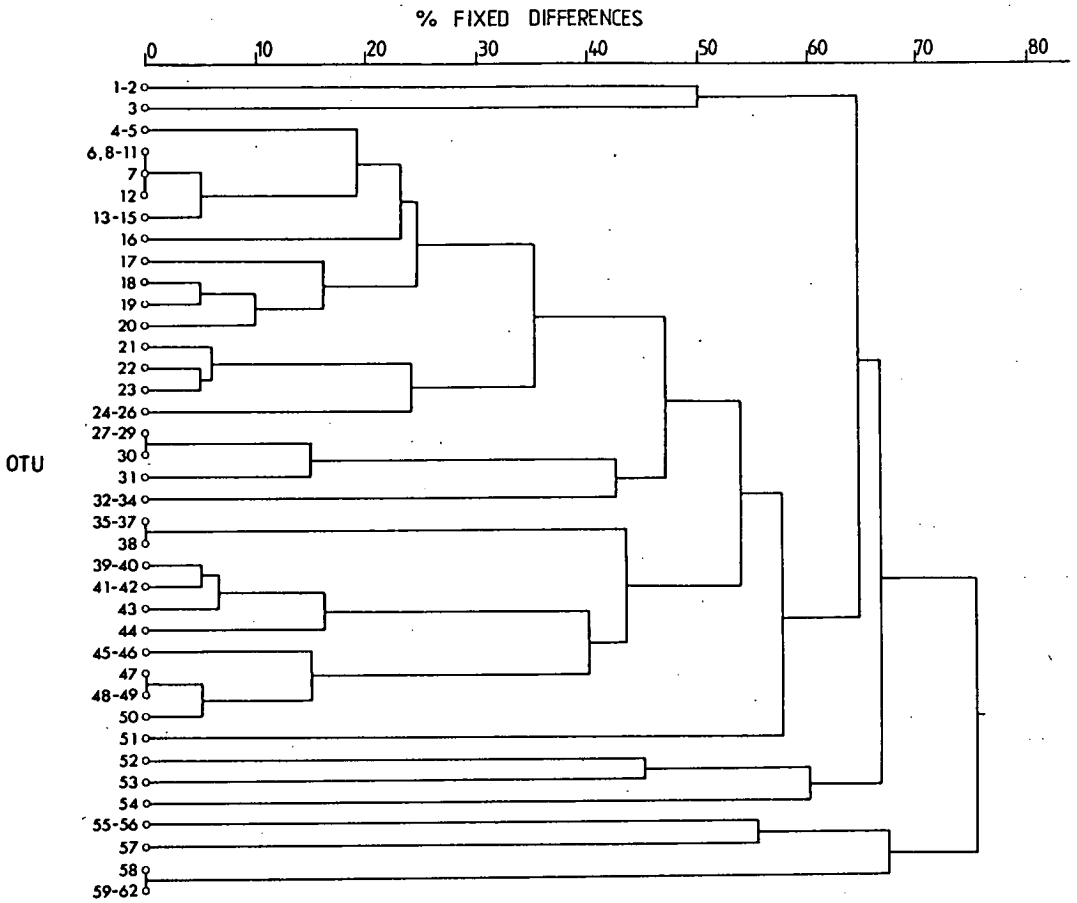
Table 9: The average estimated heterozygosities per locus per OTU (H_E), the observed heterozygosities per locus per OTU (H_O) and the proportion of polymorphic loci for each OTU (P) in the Victorian Study.

OTU	H_E	H_O	P	OTU	H_E	H_O	P
1	0.014	0.017	0.05	**32**	0	0	0
2	0.022	0	0.05	**33**	0	0	0
3	0	0	0	**34**	0	0	0
4	0.092	0.125	0.25	**35**	0.025	0.050	0.05
5	0.069	0.017	0.15	**36**	0	0	0
6	0	0	0	**37**	0.060	0.033	0.15
7	0.025	0.050	0.05	**38**	0	0	0
8	0.022	0	0.05	**39**	0.037	0.025	0.10
9	0.063	0.050	0.10	**40**	0	0	0
10	0.014	0.017	0.05	**41**	0	0	0
11	0.048	0.050	0.10	**42**	0	0	0
12	0	0	0	**43**	0.021	0.030	0.05
13	0	0	0	**44**	0.069	0.083	0.15
14	0	0	0	**45**	0	0	0
15	0.011	0.012	0.05	**46**	0.024	0.025	0.05
16	0.025	0.050	0.05	**47**	0.014	0.014	0.05
17	0	0	0	**48**	0	0	0
18	0	0	0	**49**	0	0	0
19	0.014	0.017	0.05	**50**	0.022	0.033	0.05
20	0	0	0	**51**	0	0	0
21	0	0	0	**52**	0.024	0.025	0.05
22	0.047	0.050	0.10	**53**	0	0	0
23	0.021	0.010	0.05	**54**	0.036	0.050	0.10
24	0	0	0	**55**	0	0	0
25	0	0	0	**56**	0	0	0
26	0.061	0.033	0.15	**57**	0.052	0.038	0.15
27	0	0	0	**58**	0	0	0
28	0.042	0.050	0.15	**59**	0.025	0.050	0.05
29	0.025	0.050	0.05	**60**	0.049	0.075	0.10
30	0.061	0.067	0.15	**61**	0.025	0.050	0.05
31	0.011	0.012	0.05	**62**	0.048	0.050	0.10

OTU	1-2	4-5	17	7	16	6,8-11	18	12	52	55-56	13-15	35-37	20	19	21	22	54	53	3	23	24-26	39-40	41-42	44	43	27-29	45-46	47	48-49	50	32-34	58	59-62	31	51	30	38	57
1-2		851	802	594	571	723	507	669	1233	1400	764	907	694	588	694	718	1065	1650	694	652	860	1062	1025	982	1014	1238	1071	1336	1362	1492	1106	2374	2210	1355	1222	999	924	1317
4-5	45		496	336	375	366	362	362	682	839	355	817	362	353	435	445	1087	809	908	314	574	1048	853	729	924	1068	738	951	936	870	850	1343	1321	980	1027	1152	827	1179
17	55	30		331	296	398	223	357	1236	680	419	899	163	204	492	468	1102	916	916	586	686	1111	1050	1055	904	1083	952	1412	1386	1517	833	1558	1524	1025	916	1086	916	986
7	45	20	30		236	20	167	0	880	877	51	546	262	158	405	470	1052	1024	619	382	549	702	668	711	560	574	690	1040	1024	1111	542	1531	1484	578	830	581	572	735
16	45	20	25	20		328	229	296	1050	942	356	719	229	287	377	389	1185	1265	668	298	461	1086	891	889	879	913	862	1288	1265	1382	693	1413	1369	884	830	917	719	984
6,8-11	45	15	30	0	20		247	28	819	840	48	519	309	196	415	469	1053	938	600	404	527	680	644	682	537	578	734	1012	1000	1087	522	1515	1435	602	826	597	556	776
18	40	20	20	15	20	15		223	816	680	276	674	105	52	325	389	1210	916	598	396	575	728	799	793	586	812	827	1224	1204	1309	523	1558	1509	792	916	818	693	1010
12	50	20	30	0	25	0	20		905	903	60	549	288	154	405	496	1005	1050	693	419	575	728	693	736	586	600	716	1066	1050	1137	523	1558	1509	603	799	606	598	761
52	70	45	70	60	65	50	55	60		1062	729	914	816	827	969	999	937	610	937	839	671	829	905	708	776	1293	1452	1367	1375	1585	721	1375	1470	1369	1236	1519	937	1916
55-56	75	55	50	60	60	55	50	60	65		770	1162	680	671	796	634	1617	1036	1036	891	883	1267	1190	1147	1025	1312	1450	1631	1596	1503	1225	1321	1341	1184	1036	1516	1190	886
13-15	55	20	35	5	30	5	25	5	50	55		469	317	228	326	403	1047	852	587	313	467	608	578	680	479	492	806	908	895	968	455	1319	1380	496	719	533	500	748
35-37	60	45	60	40	50	35	50	40	50	70	35		780	616	696	721	1037	984	692	680	481	407	479	703	549	898	687	477	472	573	727	1050	1237	886	642	947	13	1552
20	50	20	15	25	20	20	10	25	55	50	25	55		106	405	468	1399	916	799	442	657	840	916	915	681	938	952	1412	1386	1517	616	1558	1509	910	916	943	799	1010
19	45	20	15	15	25	15	5	15	55	50	20	45	10		316	380	1027	887	685	410	566	738	790	784	593	804	819	1216	1195	1300	443	1549	1501	781	790	809	685	1001
21	50	28	39	33	33	33	28	33	61	56	28	50	33	28		70	1103	944	588	57	385	858	811	896	680	489	1154	1306	1281	1030	504	1041	1097	581	944	528	693	769
22	50	25	35	35	30	35	30	35	60	45	30	50	35	30	6		1211	1021	681	74	288	938	769	844	758	620	1066	1195	1175	961	624	1122	1137	709	1045	659	733	788
54	65	65	65	60	70	60	70	60	60	80	60	55	75	60	67	65		936	1154	1040	1168	1673	1875	1255	1575	1459	1441	1579	1587	1495	1088	1496	1542	1491	1182	1510	1128	1794
53	80	55	60	65	70	50	60	65	45	65	55	55	60	55	61	65	60		916	943	656	967	916	960	787	732	1095	1041	1050	990	774	1558	1512	778	1204	964	1050	2274
3	50	50	60	45	50	35	45	50	60	65	45	50	55	50	44	45	65	60		652	635	945	1050	1279	904	847	1463	1378	1386	1517	774	1846	1713	1043	1204	964	693	1842
23	45	20	45	30	25	30	30	35	55	60	25	50	35	35	6	5	65	60	45		312	955	787	861	775	588	1084	1212	1192	979	604	1140	1188	675	904	626	681	866
24-26	55	30	45	40	35	30	40	40	40	60	30	35	40	40	28	20	65	40	45	20		625	504	759	493	697	718	814	802	870	452	1485	1521	650	802	795	486	1200
39-40	65	60	65	50	65	40	50	50	55	70	40	30	55	50	56	60	80	60	60	60	45		71	242	76	731	615	385	381	473	624	1532	1845	645	1111	906	439	1309
41-42	65	55	65	50	60	40	55	50	60	70	40	35	60	55	56	55	85	60	65	55	40	5		149	110	678	525	360	357	443	692	1558	1870	591	1050	857	511	1226
44	65	50	65	50	60	40	55	50	50	65	40	40	60	55	56	55	70	60	70	55	50	15	10		287	1034	655	511	510	619	1016	1448	1631	931	1487	1220	755	1314
43	65	55	60	45	60	35	45	45	55	65	35	40	50	45	50	55	80	55	60	55	40	5	10	20		567	513	505	499	543	489	1833	1949	493	904	730	586	1099
27-29	70	55	60	40	55	40	50	40	70	70	35	50	55	50	33	40	75	50	55	40	45	45	45	55	40		1098	1042	1049	987	536	1805	1722	131	1127	97	938	985
45-46	65	45	60	50	55	40	55	50	75	75	50	50	60	55	67	55	75	65	75	65	50	45	40	45	40	60		189	187	216	969	1816	1841	946	1095	1320	716	1434
47	70	60	75	65	70	55	70	65	75	80	55	35	75	70	72	70	80	65	75	70	55	30	30	35	40	60	15		0	56	1063	1558	1961	923	1195	1279	517	2354
48-49	75	60	75	65	70	55	70	65	75	80	55	35	75	70	72	70	80	65	75	70	55	30	30	35	40	60	15	0		55	1071	1558	1961	910	1204	1315	511	2274
50	75	55	75	65	70	55	70	65	80	75	55	40	75	70	61	60	75	60	75	60	55	35	35	40	40	55	15	5	5		1167	1386	1648	856	1309	1239	615	1751
32-34	65	50	55	40	50	35	40	40	50	70	35	50	45	35	39	45	65	50	50	45	35	45	50	60	40	40	60	65	65	65		1396	1436	609	640	658	803	1315
58	89	74	79	79	74	79	79	79	74	74	74	63	79	79	65	68	74	79	84	68	74	79	79	74	84	84	84	79	79	74	74		51	1883	1558	2139	999	1305
59-62	85	65	70	70	65	70	70	70	70	60	65	55	70	70	56	55	75	75	75	60	60	70	70	65	75	75	80	75	75	70	65	0		2005	1749	2144	1189	1189
31	75	60	60	45	60	40	55	45	75	70	35	55	60	50	44	50	75	50	65	50	50	45	45	55	40	5	60	60	60	55	45	84	75		1197	309	926	936
51	70	60	60	55	55	55	60	55	70	65	50	45	60	55	61	60	70	70	70	60	55	65	65	75	60	65	65	70	70	70	45	79	80	70		1222	693	1384
30	60	60	65	45	60	40	55	45	75	75	40	55	60	55	39	45	70	55	55	45	50	55	55	65	50	0	70	65	70	65	40	84	75	25	70		988	972
38	60	50	60	45	50	40	50	45	60	70	40	0	55	50	50	50	65	65	50	50	35	35	40	50	45	55	50	40	40	45	55	63	55	60	50	60		1561
57	70	60	60	50	60	50	60	50	85	55	50	75	60	60	50	50	80	90	80	55	65	70	70	70	65	55	75	90	90	80	70	74	60	60	75	60	75	

Table 10: Genetic difference matrix for OTUs used in the Victorian Study, showing % fixed differences below the diagonal and Nei's genetic distance 'D' (Nei, 1972) X 1000, above the diagonal. See text for grouping of OTUs.



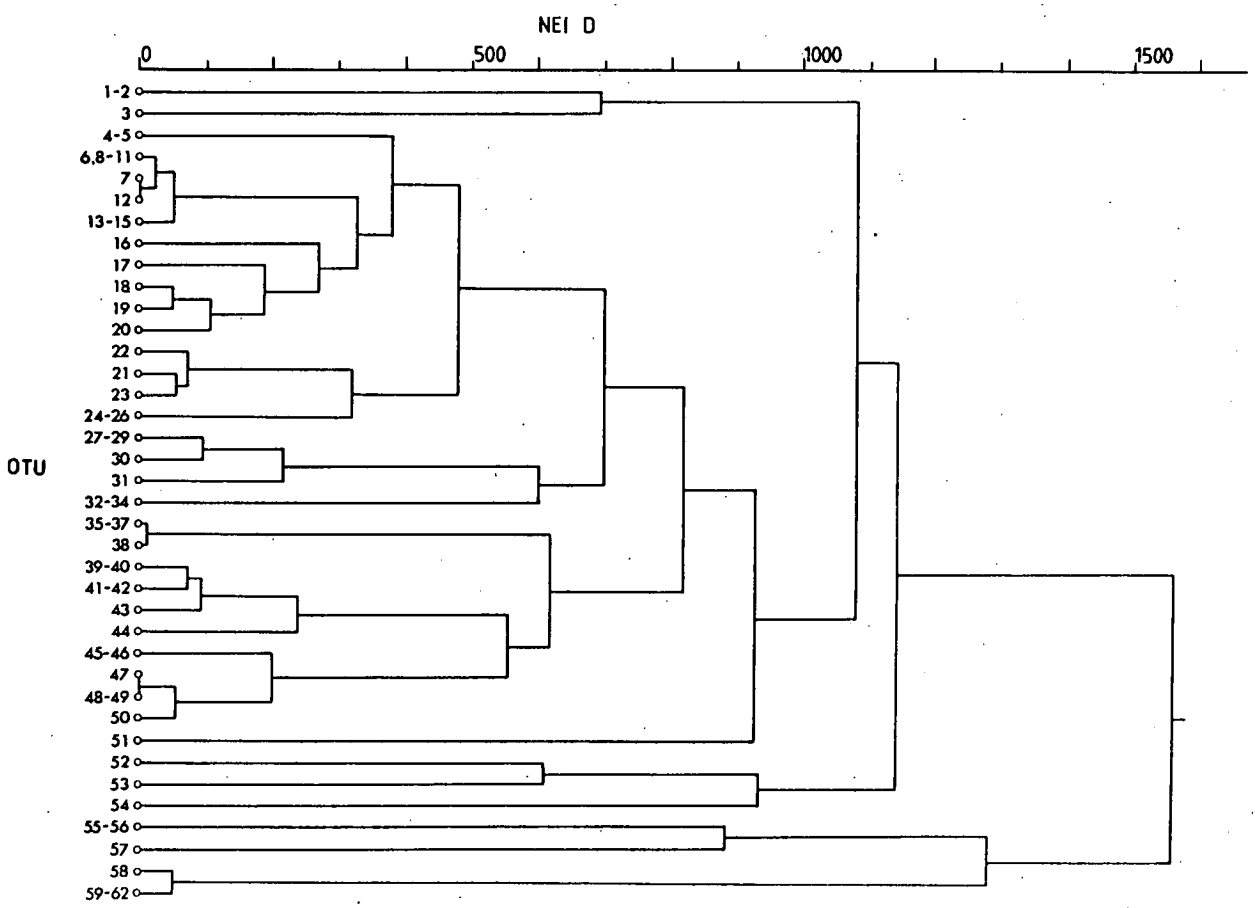


Figure 4: UPGMA clusters derived from the data presented in Table 10 showing the clusters based on % fixed differences (A) and Nei's genetic distance (B). See text for grouping of OTUs.

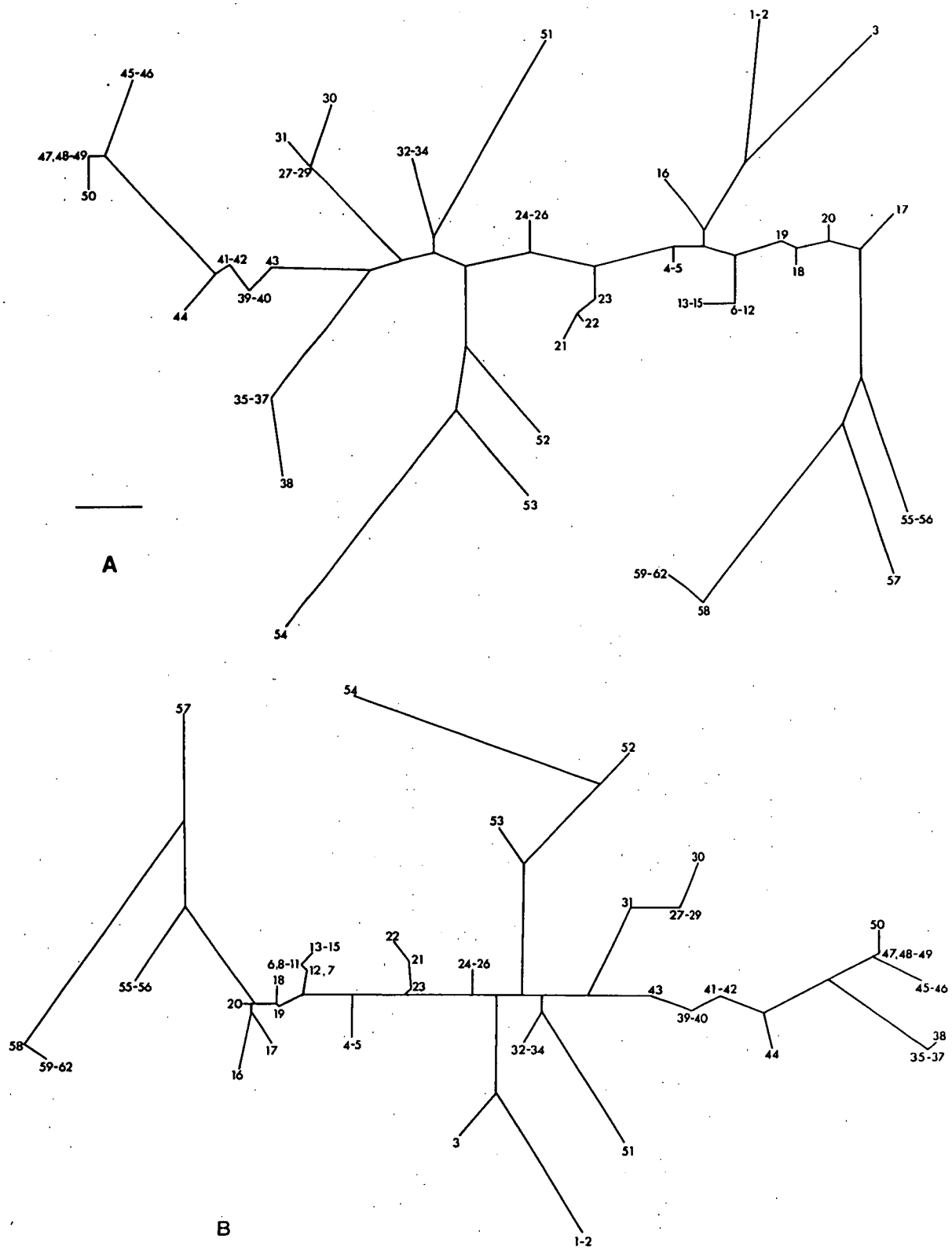


Figure 5: Wagner networks constructed from the data presented in Table 10, based on % fixed differences (A) and Nei's genetic distance (B). Scale bars represent patristic distances of 10 and 0.100 for A and B respectively. See text for grouping of OTUs.

LDH, MDH-1, PEP-B, PGK and SORDH loci) and

ii) **78** & **81** (which show fixed differences at the ADH, ALD, ENOL, GOT-1, GOT-2, LDH, MDH-1, PEP-B, PGK and PhDH loci).

The large number of fixed differences are reflected in the D's of 0.793 and 0.719 for these two pairs respectively (see Table 7).

There is no doubt that in each of these cases of sympatry, distinct species are involved; this can be demonstrated by the following calculations. OTUs **74** and **75** consist of 6 and 5 individuals respectively. The null hypothesis is that all 11 individuals come from the same random-breeding population. Then for one locus showing no heterozygotes (say GOT-1), the estimated allele frequencies are p = 10/22 = 0.45 and q = 12/22 = 0.55. If the population were in Hardy Weinberg equilibrium, the expected proportion of heterozygotes becomes 2pq = 0.50. The probability of not observing any heterozygotes in 11 individuals is $(0.5)^{11} = 0.0005$. Therefore, the absence of any heterozygotes at only one such locus would be very strong evidence for sympatric species. For 11 such loci, the probability becomes $(0.0005)^{11} = 3.7 \times 10^{-37}$, a very small probability indeed.

The same calculations for the OTUs **78** and **81** yield a probability level of 7.9×10^{-31}.

Allopatric OTUs

For the following three pairs of OTUs, the differences between each member of the pair were small:

i) **72** and **73**, where identical patterns were shown except at the SORDH locus, corresponding to D = 0.051,

ii) **78** and **79**, where fixed differences only occurred at the ENOL locus, and differing gene frequencies appeared at at least two other loci (SORDH and MDH-1) (D = 0.108), and

iii) **80** and **81**, with fixed differences at two loci (GOT-2 and GA3PD), and with shared alleles which are unique in the Tasmanian Study at the loci of ENOL (allele B), GOT-1 (allele B), MDH-1 (alleles F and H) and PEP-B (allele A) (D = 0.135).

OTUs **31** and **57** from Victoria exhibited alleles which were unique to the Study at the loci of ALD (alleles F for **57** and B for **31**), GA3PD (allele A for **57**), GOT-2 (allele H for **31**), GPI (alleles H for **57** and F for **31**), GPT (allele A for **57**), MDH-1 (allele C for **31**) and MDH-2 (alleles A for **57** and F for **31**).

Other unique alleles were found for **71** at GOT-2 (allele G) and GPI (allele A); for **74** at GPI (allele E) and GPT (allele E); for **75** at GPI (allele G) and LDH (allele B); for **76** at MDH-1 (allele E); for **77** at ENOL (allele D), GOT-2 (allele C) and TPI (allele A); for **82** at GOT-2 (allele A), MDH-1 (allele G), MDH-2 (allele E) and PEP-B (allele G); for **51** at PGK (allele A) and TPI (allele B); for **83** at GOT-1 (allele F), GPT (allele B), LDH (allele D), MDH-2 (allele C) and PEP-B (allele D); and for **84** at ALD (allele E) and MDH-2 (allele B).

In general, D's for closely-related OTUs range from 0.051 to 0.359, whilst the more distantly related values range from 0.357 to 1.897 (see Table 7). The differences

between the OTU pairs of **72** & **73**, **78** & **79** and **80** & **81** are all extremely small (for instance between 5 and 10% fixed differences) and contrast to all other OTU comparisons (which are ≥ 25%). The remaining % fixed differences and D's for OTU comparisons range from 25% and 0.289 respectively for the OTU pair **73** & **75**, to 85% and 1.897 respectively for **76** & **83**.

DELINEATION OF SPECIES - VICTORIAN STUDY

In order to reduce the data set to manageable proportions, closely affiliated OTUs were combined to form single OTUs which were then treated as homogeneous populations. The combinations were made on the basis of the information presented below.

Sympatric OTUs

The Victorian Study included nine cases of sympatric OTUs, and in each case they showed fixed differences. The OTUs, the loci at which these differences were found, and the probability levels (according to the calculations above) are given below.

i) **3** and **29**; 6PGD, ALD, ENOL, FDPase, GOT-2, IDH, LDH, MDH-1, MDH-2, MPI and PhDH; these OTUs, each of three individuals, had fixed differences at 11 loci and the probability of such a result from a random breeding population is 1.4×10^{-20}.

ii) **2**, **12** and **27**; exhibit fixed differences at the loci of 6PGD, ALD, IDH, MDH-2 and PGK; sample sizes here are 3, 3 and 3 respectively. OTUs **2** and **12** differ at 10 loci ($p = 8.7 \times 10^{-19}$), **2** and **27** at 14 loci ($p = 5.2 \times 10^{-26}$) and **12** and **27** at 8 loci ($p = 3.6 \times 10^{-15}$).

iii) **5** and **25**; exhibit fixed differences at the loci of 6PGD, ENOL, GOT-1, GPI, MDH-1, MDH-2, PhDH and SORDH; sample sizes are 3 and 2 respectively ($p = 1.8 \times 10^{-13}$).

iv) **7**, **16**, **17** and **57**; sample sizes here are 1, 1, 1 and 4 respectively, where OTUs **7** and **16** differ at 4 loci (ADH, FDPase, GOT-2 and GPT; $p = 3.9 \times 10^{-3}$), OTUs **7** and **17** differ at 6 loci (6PGD, ADH, FDPase, GOT-2, IDH and GPT; $p = 2.4 \times 10^{-4}$), OTUs **16** and **17** differ at 5 loci (6PGD, GOT-2, IDH, MDH-1 and TPI; $p = 9.8 \times 10^{-4}$), OTUs **7** and **57** (at 10 loci, 6PGD, ADH, ALD, FUM, GA3PD, GPT, LDH, MDH-1, MDH-2 and IDH; $p = 1.8 \times 10^{-25}$), OTUs **16** and **57** (at 12 loci, 6PGD, ADH, ALD, FUM, GA3PD, GPT, IDH, GOT-2, LDH, MDH-1, MDH-2 and FDPase; $p = 2.0 \times 10^{-30}$) and OTUs **17** and **57** (at 12 loci, ADH, ALD, FUM, GA3PD, GPT, IDH, LDH, MDH-1, MDH-2, GOT-2, FDPase and TPI; $p = 2.0 \times 10^{-30}$).

v) **11** and **46**; exhibit fixed differences at the loci of 6PGD, ENOL, GA3PD, GOT-1, GOT-2, IDH, MDH-1, PhDH, SORDH and TPI; sample sizes are 2 and 2 ($p = 9.1 \times 10^{-13}$).

vi) **9** and **53**; exhibit fixed differences at the loci of ALD, ENOL, FDPase, GOT-1, GOT-2, IDH, GPI, GPT, LDH, PGK and TPI; sample sizes are 3 and 3 ($p = 1.4 \times 10^{-20}$).

vii) **8** and **33**; exhibit fixed differences at the loci of ENOL, FDPase, GOT-2, GPI, GPT, IDH, MDH-1, MDH-2 and PGK; sample sizes are 3 and 2 respectively ($p = 4.5 \times$

10^{-15}).

viii) **39** and **41**; sample sizes for these two OTUs were 4 and 3 respectively, with one fixed difference at the GOT-2 locus; nevertheless the probability is 3.0×10^{-3}; the two OTUs also showed major allele frequency differences for MPI and GPI.

ix) **47** and **50**; sample sizes were 3 and 3, with one fixed difference at the LDH locus, giving a $p = 1.6 \times 10^{-2}$, showing that it is statistically possible that these 6 individuals come from the same population and that by chance no heterozygote was sampled in the 6 individuals. Evidence against this, however is provided by the SORDH locus where **50** possessed allele c at a frequency of 0.33, but this allele was absent from **47**.

The lowest level of D for two sympatric species was 0.056 (for OTUs **47** and **50**), a figure which is only marginally lower than the lowest level in the literature for freshwater decapods. However, most differences between sympatric species were well above this level in this study, as were species differences in the literature (see Table 1).

For sympatric OTUs, in by far the majority of cases, the genetic distances were large and merely served to substantiate the original impression that the OTUs actually represent different species. Probably the real value of identifying fixed differences in sympatry comes from the comparison of very closely related species, in fact where the species concerned are their closest living relatives. Overall, on three occasions all in the Victorian Study, the OTUs in sympatry exhibited differences which were around, or lower than 0.300. At site V80, the three OTUs **7**, **16** and **17** exhibited D's of 0.236 (**7** and **16**), 0.296 (**16** and **17**) and 0.331 (**7** and **17**), at site V03 for the OTUs **47** and **50** (D = 0.056), and at site V09 where D = 0.071 was found between **39** and **41**.

Allopatric OTUs

In contrast to the Tasmanian Study, the variation between the OTUs in the Victorian Study spans the spectrum of very small to very large D's and % fixed differences, presented both easily delineated OTUs and some difficult groups of OTUs.

The single OTUs **3**, **52**, **53**, **54**, **57** (and **51** from Tasmania) are all clearly separated from each other and from all other OTUs in the Victorian Study (see Table 10). The OTU pair of **1** and **2** exhibit only one fixed difference (at the PGK locus) and D = 0.054, and since they are clearly different to all other OTUs, they will be considered as conspecific, as should **4** and **5** since they showed no fixed differences and D = 0.064. The OTUs **55** and **56** were identical at all loci except for a fixed difference at GOT-1 and displayed three alleles which are unique for the Study (namely allele A at the ALD locus, allele F at the GOT-2 locus, and allele A at the LDH locus); these two OTUs will be considered as conspecific.

OTUs **6-26**

Probably the most complex set of relationships are found for the OTUs **6-26**. They can be subdivided into 5 and these groups suggest probable species break-ups.

The OTUs **6**, **7**, **8**, **9**, **10**, **11**, **12**, **13**, **14** and **15** exhibited only 5 variable loci between them (6PGD, GPI, GPT, PGK and PhDH), and overall fixed differences were only found on two occasions (at locus PGK for OTU **6**, and at locus GPT for OTUs **13**, **14**

and **15**). Table 11 gives the % fixed differences and genetic distances between these OTUs, and shows D's which range from 0.000 to 0.173; these groups thus form a relatively succinct group. The most distantly related OTUs are **6** and **15**; the OTU pairs 7 and **12**, and **13** and **14** are identical. The group includes OTU 7 from the sympatric site V80, and if these OTUs are together considered to represent a discrete species, then this sympatric occurrence separates them from the OTUs **16** and **17** at least.

OTU **16** displays D's of always more than 0.229 and this information coupled with the fixed differences in sympatry with **7** and **17** suggests that it may well be considered as a distinct species, but this status needs to be verified by additional information. OTU **16** expresses allele E at the MDH-1 locus, an allele which is unique in the Victorian Study.

The most likely scenario for the OTUs **17-20** is for the group to be considered as conspecific, since they show close affinities at the variable GOT-2 and IDH loci. Whilst the OTUs **18-20** are very closely affiliated (with a maximum D between them of 0.106; see Table 10) the relationships between **17** and **18-20** are somewhat more obscure, with D's of around 0.200; this needs to be further investigated.

The OTUs **21-23** appear to be closely affiliated (see for example the LDH and MDH-1 loci) and D's for this group do not exceed the level of 0.074. The closest group of OTUs to them is **24-26** with a D-value of 0.288 (see Table 10). There is thus strong evidence to suggest that **21-23** should be considered as conspecific.

Similarly, the OTUs **24-26** display D's between them ranging from 0.051 - 0.080, and they appear to be succinct (see for example, the MDH-1 and PhDH loci). The closest extra-group affinity is with OTU **22** (see above).

OTUs **27-31**

The OTUs **27, 28, 29, 30** and **31** appear to be very closely related and they form a discrete group. They share alleles at the loci of ALD (allele B) and MDH-2 (alleles J and K) which are unique in the Study. Within the group, OTU **31** exhibits a fixed difference at the MDH-1 locus (allele K which is unique in the Study) plus the occurrence of an original allele at the GOT-2 locus; OTU **30** displays original alleles at the FUM, GPT and PGK loci. Table 12 gives the individual relationships within the group, showing that the largest differences are found between **31** and either **29** or **30**.

There is thus strong evidence to suggest that the OTUs should be considered as conspecific (see Discussion, under Geographical Variation).

OTUs **32-34**

Whilst the OTUs **32, 33** and **34** show 4 fixed gene differences amongst themselves (at the loci FDPase, MDH-1, PGK and PhDH), their apparent closeness to each other and sufficient difference to the rest of the OTUs (see for example the occurrence of allele E at the MDH-2 locus and allele E at the IDH locus which are not found in any other OTUs in this Study) suggest that they can be lumped together. The OTUs clearly belong together but fixed differences of 15 % between them warrant subgroup examination, particularly since the geographical distances between these OTUs is large.

OTUs 35-38

These OTUs form a group which is distinct from others in this study (see for example the loci of MDH-1 and MPI in Table 8 and the D's in Table 10). Within the group no fixed differences were found and D's ranged from 0.004 (between **37** and **38**) to 0.111 (between **36** and **38**).

OTUs 39-44

Whilst the OTU group **39-44** incorporates two sympatric species (**39** and **41**) the respective relationships within the group are too complex to allow distinct categorization; for this group the sympatric pair will be recognised as distinct species, but the remaining OTUs will be assigned to their statuses only after additional information has been examined. The group is relatively homogeneous (see for example, the 6PGD and MDH-2 loci). Within the group, the OTU **44** is the most widely divergent, exhibiting fixed differences at the ENOL and GOT-1 loci and unique alleles to this group at the FDPase, PGK and GPT loci. Similarly, for **43** a fixed difference occurred at the SORDH locus and unique alleles to the group were found at the MDH-2 locus. Of the remaining four OTUs in the group, **39** and **41** showed fixed differences in sympatry (see above), and the evidence from the GOT-2 locus suggests that **39** and **40** could be related, and that **41** and **42** could be related.

OTUs 45-50

The relationships between the OTUs in this group are close (see for instance the 6PGD, GOT-1 and MDH-1 loci). Nevertheless they are easily resolved into proposed species. The group includes the sympatric species **47** and **50**; in addition **47, 48** and **49** exhibit only very small differences ($D < 0.050$). The OTUs **45** and **46** exhibit many similarities including the same distinct alleles at the GPT and IDH loci, and whilst they do display one fixed difference at the MPI locus, are closely related to each other ($D = 0.083$), but more distantly related to the other OTUs ($D > 0.180$). Provided that extra information can be found to support this data, this group should therefore be considered to include three species comprising **47-49, 50** and **45-46**.

OTUs 58-62

These share three alleles which are unique in the Study (namely allele D at the ADH locus, allele F at the GOT-1 locus and allele E at the PhDH locus) and otherwise form a relatively tight group. In general the OTUs **58** and **59** are closely affiliated, as are **60** and **61** (see Table 13). OTU **60** exhibits a fixed difference at the TPI locus, and with **61**, a fixed difference from **58, 59** and **62** at the GPT locus. The largest differences occur between **60** + **61** and **58** + **59** ($0.137 < D < 0.217$), and between the OTUs **60** and **62** ($D = 0.246$). This group is discussed further in the Discussion, under Geographical Variation.

	6	7	8	9	10	11	12	13	14	15
6	X	55	117	163	112	84	54	111	111	173
7	5	X	51	69	56	28	0	55	55	85
8	10	5	X	22	98	3	58	58	58	79
9	10	5	0	X	91	44	101	101	101	82
10	10	5	5	0	X	70	55	102	102	164
11	5	0	0	0	5	X	27	41	41	76
12	5	0	5	5	5	0	X	54	54	112
13	10	5	5	5	10	5	5	X	0	54
14	10	5	5	5	10	5	5	0	X	54
15	15	10	5	5	15	10	10	5	5	X

Table 11: Showing the % fixed differences below the diagonal and the genetic distances (D x 1000) above the diagonal for the OTUs **6-15**.

	27	28	29	30	31
27	X	36	188	224	112
28	0	X	103	133	135
29	5	5	X	28	292
30	15	5	0	X	309
31	10	5	25	25	X

Table 12: Showing the % fixed differences below the diagonal and genetic distances (D X 1000) above the diagonal, for the OTUs **27-31**.

	58	59	60	61	62
58	X	40	191	217	86
59	0	X	204	137	84
60	15	15	X	63	246
61	10	10	5	X	57
62	5	5	10	5	X

Table 13: Genetic difference matrix for the OTUs **58-62** showing the % fixed differences below the diagonal and genetic distances (D X 1000) above the diagonal.

Discussion

DELINEATION OF SPECIES

The information resulting from the Tasmanian Study presents a relatively clear-cut picture and one which is easily resolved into species groups. The sympatric OTUs **74** and **75**, from north-east Tasmania, and **78** and **81** from north-west Tasmania, represent distinct species at their respective sites. For allopatric OTUs there was a discrete difference between closely related OTUs (representing the same species) and more distantly related OTUs (representing different species). The only group of OTUs to present a slightly confused picture was **72, 73** and **75**; here the OTUs **72** and **73** are apparently conspecific (D = 0.51) and **72** and **75** are separated by a D of 0.359. However, **73** and **75** have D = 0.289; this information shows that there is strong evidence to combine **72** and **73** but separate **75**, but information will have to be examined from other sources to settle the question.

The list below therefore represents the OTUs which can be delineated as species (where OTUs in brackets represent control OTUs from the other Study, and the asterisk represents a group for which not enough evidence has been provided to separate the OTUs):

71
***72, 73 and 75**
74
76
77
78 and 79
51
80 and 81
82
83
84
(57)
(14)
(31)

In the Victorian Study, at nine sites of sympatry, distinct species were identified. For allopatric OTUs, probable species boundaries were defined after consideration of factors such as the amount of genetic variation both between and within previously identified species (from the literature and sympatric sites in this thesis), and the geographical pattern of the variation for widespread groups. Consequently, the following list was compiled; it consists of either a single OTU or groups of OTUs, each of which was considered to represent a distinct species (where OTUs in brackets represent control OTUs from the other Study and asterisks highlight groups which are inconclusively delineated).

1-2
3
4-5
***6-26: 6-15, 16, 17-20, 21-23, 24-26.**
27-31
32-34
35-38
***39-44**
***45-50: 45-46, 47-49, 50.**
(51)
52
53
54
55-56
57
58-62

RELATING THE TWO STUDIES

The splitting of the work into two Studies has created two theoretical problems:

i) that conspecific OTUs, if unknowingly represented in each Study, might be recognised as separate species, and

ii) that the relationships and affinities between the Tasmanian Study and the Victorian Study are somewhat obscured.

The first of these problems is much more easily resolved than the second, since measures were taken to avoid the duplication of taxa prior to the analysis. In the case of 3 OTUs from Tasmania (**13, 14** and **15**), a morphological appraisal of the specimens suggested that there was a probable congruence with some Victorian OTUs; the examination of this group of OTUs was therefore performed in the Victorian Study in toto, and a representative from **14** was included in the Tasmanian Study for comparative purposes. In all other cases, no close morphological congruence was found between specimens in the two Studies so they were assumed to represent different taxa (at the specific, not necessarily supraspecific or subgeneric, level).

In addition, since a large subset of alleles were held by the OTUs in common for both Studies, the alleles for each loci could be interrelated between the two Studies and a qualitative comparison between the Studies was made to ensure that similar patterns of allelic variation did not occur.

One way to test for the phylogenetic affinities between the Tasmanian OTUs and the Victorian OTUs could be to use an 'Hennigian approach' to identify plesiomorphic and apomorphic alleles for easily interpreted loci and construct a qualitative phylogeny (as described by Patton and Avise, 1983). However, such an approach could only have been undertaken if a common outgroup had been incorporated into each Study.

Instead, two less effective approaches to this problem can be made, namely to examine the occurrence of unique alleles for the OTUs in common to both studies, and simply to examine the position of these OTUs in the phylogenies presented in the Wagner Trees and phenograms.

The OTUs **31, 51** and **57** display unique alleles, and each of these alleles can be described as being either unique to the overall study of *Engaeus* or unique only to one Study and not to the other; if the latter is the case, then one can assume that the OTU holds less affinities to the Study in which it has the unique allele, in fact the more unique alleles it expresses, the stronger this assumption will become. For instance, the Tasmanian OTU **51** expresses one allele at the TPI locus which is unique to the genus *Engaeus*, one allele which is unique only to the Tasmanian Study (PGK, allele A), and two alleles which are unique to the Victorian Study (FUM, allele B; GOT-2, allele B); in the Tasmanian Study the allele at the FUM locus can be found for three other OTUs. OTU **51** can therefore be assumed to have closer affinities to Tasmanian OTUs than to Victorian OTUs. OTU **57** exhibits 4 unique alleles in the Victorian Study, but 5 in the Tasmanian Study and can therefore be assumed to have closer affinities to Victorian OTUs than Tasmanian OTUs. The same applies for **31**,

where the closest living relatives are found in Victoria (even by excluding its conspecifics). It is interesting that OTU **14** has no unique alleles in either study.

Secondly, the OTUs **14**, **31** and **57** in the Tasmanian Study cluster together before they are included in the cluster of the Tasmanian species. In addition, the OTU **51** is incorporated distantly in the UPGMA for the Victorian Study.

This information suggests that, in general, at the supraspecific level, Tasmanian species appear to show a closer affinity to each other than they do to the Victorian species (including OTUs **14** et al.) and vice versa, especially for the cross-referenced OTUs. This suggestion needs to be substantiated with more conclusive data.

HETEROZYGOSITIES

Nei and Graur (1984) examined a wide range of electrophoretic data and concluded from them that invertebrates had a higher average level of heterozygosity when compared to the average level for vertebrates, but that within the invertebrates considerable variation exists. This can be shown by examining the orders of the Crustacea. The orders of Diplostraca, Copepoda, Cirripedia and Euphauseacea have much higher levels of genetic variation (with average heterozygosities of ≥ 0.12) than remaining forms (Hedgecock et al., 1982). In particular the decapods are earning a reputation for repeatedly displaying extremely low levels of heterozygosity (see for example, Tracey et al., 1975; Mulley and Latter, 1980; Nelson and Hedgecock, 1980). Hedgecock et al. (1982) suggested that within the Crustacea there was a general trend for higher levels of genetic variation for forms with relatively small body size or extremely low mobility. Other authors (such as Nei and Graur, 1984) have suggested that levels of heterozygosities are not related to environmental factors but are much more dependent upon factors such as the effective population size.

In this study the levels of the average estimated heterozygosity per locus per OTU were low and conformed to the standard trend for decapods, ranging from 0.0 to 0.092 and never exceeding 0.10. The overall averages were 0.0208 for the Victorian Study (but excluding the OTUs **13**, **14**, **15** and **51** from Tasmania) and 0.0106 for the Tasmanian Study (including **13**, **14** and **15**, and excluding the Victorian OTUs **31** and **57**). The average heterozygosities were not corrected for small sample sizes since the analysis incorporated a large number of loci, and, because all heterozygosities were very low, the estimated and observed heterozygosities were unlikely to be significantly different from the corrected values (Nei, 1978). The difference between the Tasmanian OTUs and Victorian OTUs is not significant ($t_{(df=74)} = 1.695$, $0.10 > p > 0.05$).

A comparison with the overall average heterozygosities for decapods capable of inhabiting freshwater in Table 1 reveals that the levels found here are closest to those found for the freshwater crayfish genera *Procambarus* (Astacidae) and *Cherax* (Parastacidae).

One possible explanation for this low level could be the omission from the study of notoriously variable enzymes (Gorman and Renzi, 1979) such as the esterases or phosphoglucomutase (see for example Fuller and Lester, 1980; Mulley and Latter, 1981a and 1981b). However, other variable enzymes have been included in this study, for instance MPI

(see Boulton and Knott, 1984) and MDH-1 (see Nemeth and Tracey, 1979). This explanation is therefore considered to be unlikely.

Two opposing views in population genetics state that polymorphisms are either

i) transient states in an evolutionary process governed by simple laws of probability (the 'neutral selectivity' of alleles), or

ii) adaptive responses produced by the action of selective pressures from the environment (the 'balancing selection' of alleles).

According to the neutral theory, the theoretical amount of neutral variation or polymorphism in a population depends upon both the rate at which new alleles or mutations appear in each generation and the effective size of the reproducing population. With this in mind, Hedgecock et al. (1982) proposed 2 possible ways to account for the low level of heterozygosity in decapods. Firstly, the effective population size may be far below the number of reproductively-active adults due to unequal sex ratios, or secondly, drastic reductions of population size may force the populations through a bottleneck and thus reduce genetic variability. These effects of bottlenecks can be extremely long-lived in a population (Chakraborty and Nei, 1977).

For *Engaeus* the first of these explanations is not likely to cause the low levels since sex ratios are almost always equal (see Life History Notes for each species in Chapter 6). The second explanation is more likely, since restricted geographic ranges are commonplace for the genus *Engaeus* (see Chapter 4), thus either reducing the theoretical population size or increasing the possibility that the population has passed through a bottleneck in the past. For instance the species **77, 51** and **74** in the Tasmanian Study and **3** and **53** from the Victorian Study all show extremely low levels of heterozygosity and have all been found in very restricted geographical localities. On the other hand species covering a wider geographical range in general exhibit higher (but still low) levels of heterozygosities, for instance **27-31, 58-62** and **6-15**. Within the group **6-15** the populations **13-15** have reduced levels and are geographically isolated from the remaining populations, occurring on Flinders Island and on the Tasmanian mainland where its numbers are low. The occurrence of bottlenecks in the genus *Engaeus* might therefore contribute to the low level of heterozygosity, but this needs to be investigated further. In a possible correlate of the above suggestion, Fuller and Lester (1980) examined the genetic variability of *Palaemonetes pugio* in landlocked ponds and compared them to populations attributed to a general panmixia, concluding that small, isolated populations are less genetically variable than large interconnected populations.

According to the theory of balancing selection, heterozygotes are maintained in a population through one of three mechanisms

i) heterosis, or overdominance,

ii) frequency dependent or apostatic selection, or

iii) selection over time or space giving alternative genotypes added advantage.

There is very little evidence to support any of these mechanisms in the literature for crustaceans, especially decapods (Hedgecock et al., 1982).

Selander and Kaufman (1973) suggested that larger, mobile organisms (particularly vertebrates) perceive their environment as 'fine-grained' or stable and predictive, developing a generalized adaptation characterized by a low genetic variability. Nelson and Hedgecock (1980) expanded this theory and for a large number of decapods observed a positive correlation between measures of environmental variation and Group I enzyme polymorphism, and a positive correlation between Group II enzyme heterozygosity and measures of trophic generalism (where 'Group I' includes central metabolic enzymes and 'Group II' includes enzymes functioning in peripheral metabolic pathways with a variety of substrates; sensu Gillespie and Kojima, 1968). They concluded that because decapods were likely to conform to a fine-grained strategy and were likely to be trophic specialists, then both Group I and II enzymes will show low levels of heterozygosity. However, most species of *Engaeus* are confined to burrows for most if not all of their lives and are largely immobile with a low vagility, therefore they are likely to perceive their environment as being coarse-grained; the model of Nelson and Hedgecock therefore predicts a higher level of heterozygosity for Group I enzymes (which has not been found).

It is therefore suggested that of the recent explanations for low levels in heterozygosity, the possibility of species passing through bottlenecks in the past seems to be the most plausible for the genus *Engaeus*.

GEOGRAPHICAL VARIATION

Changes in gene frequencies over a species' geographical range has frequently been used to define regional variation, subpopulations or clines. In general, the geographical variation among conspecific populations of decapod crustaceans can be described as low (Hedgecock et al., 1982), with the resultant effect that the heterozygosities and gene frequencies of a population are generally representative of the entire species (provided that the population has not come from an isolated site or ecologically marginal habitat; Hedgecock et al., 1982). Most studies of the geographical variation of decapod species have centred around the delineation of stocks or subpopulations for fishery purposes, for instance the penaeid prawns in Australia (Mulley and Latter, 1981a and 1981b; Richardson, 1982).

The geographical variation of species needs to be identified to prevent the delineation of OTUs which are merely variants rather than separate biological species, and this was demonstrated for some OTUs in this study. Clines (as defined by Mayr, 1963) offer the best example of possible confusions since specimens from either end of a cline may be sufficiently different from each other to warrant separate specific status; such a situation can only be avoided by the recognition of geographical intergrades.

Several of the more variable species in this work exhibit either simple allelic variation over their geographical range or what appear to be organized clinal properties.

The most obvious of these is for the OTU group **27-31** from South Gippsland, Victoria, where the OTUs are arranged in a west to east order of **31 - 27 - 28 - 29 - 30**. Examination of D's between these OTUs shows that **31** and **27** are close, as are **29** and **30**, with the central population **28** equidistant from both western and eastern populations (see

Table 12). The loci which contribute to this trend are GPI, LDH and MDH-1. For instance, at GPI, the western populations are fixed for allele F, the eastern populations are fixed for allele D and the central population expresses both alleles.

The OTUs **13-15** show extremely close affinities in the **6-15** group. **13** and **14**, from Flinders Island and north-eastern Tasmania respectively, are identical, and **15** from north-western Tasmania shares a common fixed allele at the GPT locus. This indicates that the Tasmanian members of this group are more closely related to each other than they are to the other populations. In addition, the comparatively large differences between **6** (from the Otway Ranges) and the remainder of the group might simply reflect its geographical isolation from the populations in South Gippsland, Flinders Island, and Tasmania; these differences are largest between **6** and **15**.

The group **58-62** appears to contain western, central and eastern components to its overall distribution. The populations **60** and **61** were collected from the Grampian Ranges in western Victoria, and these show very close affinities; similarly **58** and **59** from central Victorian populations exhibit close affinities. The eastern populations in this group are represented by **62** and the results show a close affiliation between it and the central populations, and a more distant relationship with OTU **60**; however, the relationship between **61** and **62** is close and this thus represents an anomaly of the above assessment.

The group **35-38** is predominantly homogeneous; one exception is at the 6PGD locus where each OTU can be arranged along a north-south gradient, the most southern population **36** expressing only allele E, whilst **38** is fixed for allele F. The central populations exhibit heterozygotes.

The evidence for geographic variation within the OTUs **32-34** is inconclusive from this study; further investigations should examine more populations from its extensive range and include individuals from Tasmania.

In Tasmania, the OTUs **78** and **79** appear to be conspecific; the variation between these two OTUs is similar to that found between Hunter Island and Dip River (see Pilot Study, Appendix II).

Very few cases of the geographical variation of allelic fequencies have been documented in the freshwater decapod fauna. Austin (1979) records preliminary information on the geographic variability of *Cherax quinquecarinatus*, showing that for 5 populations in the south-west corner of Western Australia, the northern most and the southern most populations show the greatest divergence.

Nemeth and Tracey (1979) record an apparent shift in the frequency of leucine aminopeptidase alleles in *Orconectes propinquus*, with central populations exhibiting slower alleles than either the eastern or western populations; the authors, however, did not identify this variation.

As for protein polymorphisms, clines can be explained as being either manifestations of randomly occurring shifts in allele frequencies on a regional basis, or they can be the selective products of environmental influence. If the latter, clines can imply that either the

organism has sought a particular habitat to match its genotype (which is unlikely, particularly for animals with low vagility such as *Engaeus*), or its genotype has undergone evolutionary change through selective pressure applied from different habitats (and this hypothesis is difficult to prove since one must demonstrate that selective pressure actually acts at the locus which shows the clinal properties). The occurrence of intraspecific allelic frequencies which show apparent clinal properties such as in the case of the OTUs **27-31**, is a promising area of research; by documenting habitat parameters and by performing crossbreeding experiments, perhaps the mechanism which drives the clines can be elucidated.

PHYLOGENY

There have been numerous studies which have used a set of electrophoretic data to infer the phylogeny of the organisms under investigation. In general, two approaches can be made, either phenetic or cladistic. A phenetic approach infers a phylogeny on the basis of overall similarities, using characters which are not usually weighted and never interpreted as belonging to a derived or ancestral state. A cladistic approach however infers that two taxa which share a derived character state are more closely related to each other than either is to any other taxon which is without that character state.

When large electrophoretic data sets are created, numerical methods are often employed to synthesize the data to produce a phylogeny under one or other of the approaches outlined above.

However there has been no consensus as to the appropriate method of treatment of these data (Buth, 1984), probably because there can be no such thing as an all purpose method for inferring phylogenies (Felsenstein, 1982), particularly as homoplasy occurs as the amount of time since the separation between taxa increases. The most commonly used numerical methods are the phenetic unweighted pair group with arithmetic means (UPGMA; Sneath and Sokal, 1973), the phylogenetic tree of Fitch and Margoliash (1967) and the modified Wagner tree procedure of Farris (1972) (Ferguson, 1980). The pros and cons of these techniques and the measures of distance to use are discussed in detail in Felsenstein (1982), and compared in other articles, for instance in Swofford (1982), Prager and Wilson (1978), Nei et al. (1983) and Tateno et al. (1982) and these are summarized in Buth (1984). It is not the aim of this work to pursue this argument, merely to heed its major points; both a phenetic technique and a phylogenetic technique were employed here.

Previous electrophoretic studies of decapod crustaceans have used either or both phenetic and cladistic techniques to explore phyletic relationships. For instance Boulton and Knott (1984) used electrophoresis to examine the relationships of 5 species of shrimp from an estuary at Perth, Western Australia; the phylogenetic implications (using both a UPGMA phenogram and an unrooted Wagner Tree) of the study as well as an appraisal of the morphological characters, caused the authors to cast serious doubt on the generic statuses of at least two of the species. Nemeth and Tracey (1979) examined the inter- and intrageneric differences for six species of the freshwater crayfish genera *Orconectes* and *Cambarus*, concluding that the plot of a Wagner tree based on electrophoretic data corresponded to

clustering using morphological data.

Using a different technique, the so-called 'Hennigian' approach to phylogenetic analyses, Albrecht and von Hagen (1981) specified alleles as either ancestral or derived and by the recognition of shared derived character states were able to construct the phyletic relationships of six species of freshwater crayfish belonging to the European Astacidae. The authors claimed to have congruence between this phylogeny and a phenetic one constructed along morphological grounds. Recently, however, Richardson et al. (1986) have been critical of this method.

The relationships of species and their grouping into supraspecific groups and higher levels, will not be attempted here; however the following points concerning the phylogeny of the *Engaeus* group can be made from the two clustering techniques. It must be stressed that only close affinities will be interpreted, especially where there is agreement between the phenetic and phylogenetic clusters; a more complete phylogeny for the group will be attempted in Chapter 3 where this information will be assimilated with morphological characters to produce a hypothetical phylogeny, one which can then be examined by more stringent techniques such as DNA sequencing.

Tasmanian Study

Both UPGMA clusters (Figure 2) present the same combination of OTUs and both exemplify the order of magnitude between the closely related OTUs and the more distantly related ones.

The Wagner Trees are presented in Figure 3 for % fixed differences (A) and Nei's genetic distance (B), and a relatively close correlation between the phenetic and the phylogenetic methods are obtained. Essentially, four major groups could be elucidated from them:

I **72, 73, 75** and **76**

II **51, 77** and **84**

III **80, 81** and **82**

IV **14, 31** and **57** (the OTUs incorporated from the Victorian Study).

OTUs **74, 78** and **79** are clustered with group I (above) in both phenograms and the Wagner Tree (B), but appear to be closer to group II in the Wagner Tree (A). In both trees, however, they are centrally located.

The OTUs **71** and **83** each seem markedly different to other OTUs; **83** appears to be closest to **14** but almost nothing else, and **71** is nearest to **76**; the phylogenetic relationships of these two OTUs cannot be interpreted from these results.

Species from Tasmania are in general more closely related to each other than they are to Victorian species; with **72-76, 78** and **79** showing close affinities, with the probable inclusion of **71** and **51, 77** and **84** at a higher level. The major exceptions to this dictum are **6-15** (this study) and **32-34** (Chapter 4, this volume) which can both be found in Tasmania, and the OTUs **80-83** which cluster outside the inclusion of the Victorian species in the Tasmanian Study. The interesting relationship between the OTUs **32-34** and **51** in the

Wagner Trees of the Victorian Study, where they share a common hypothetical ancestor which, according to the dictum above, suggests that the Tasmanian group as a whole and **32-34** may share a common ancestor, clearly needs to be investigated.

Victorian Study

In the UPGMA clusters (Figure 4), apart from the difference in scale, there is very little difference between the relationships presented in A and B; only a few minor structural differences exist. The positions of **21**, **22** and **23** switch so that in Figure 4 (A) **22** and **23** are more closely related, whilst in Figure 4 (B) **21** and **23** are more closely related. The OTU clusters of **6-15** and **18-20** are depicted in the same way in both figures yet subsequent affinities are slightly different; in Figure 4 (A) the former cluster progressively adds **4-5**, then **16**, whilst the latter cluster adds **17** and then the two clusters combine; but in Figure 4 (B) the latter cluster progressively adds **17**, **16**, then **6-15** and finally **4-5**.

Similarly, the Wagner networks A and B presented in Figure 5 show basically the same structure and relationships; only the relative positions of **1-2** and **3**, **16**, and **35-38** have been altered.

Figure 5 confirms the close relationships between **6-15** and **17-20**, with the OTUs **16** and **4-5** close neighbours. The groups of **21-23** and **24-26** are closer to each other than they are to any other OTU, centrally located with respect to the other OTUs and they show close affinities to the group **4-20** (but more specifically **4-15**).

Similarly, the OTUs **39-44** are tightly grouped and centrally, closely linked to the OTU group of **45-50**, then grouped with **35-38** and **27-31** more distantly.

The OTUs **32-34** are closest to **43**, **24-26** or **51** and their affinities are obscure.

OTUs **52-54** appear distantly related and are closer to each other than they are to their nearest neighbours **32-34** or **24-26**.

Finally the OTUs **55-56** and **57** appear distantly related and are closer to each other than they are to their nearest neighbours **17-20**. **58-62** are widely separated from the remaining OTUs.

In general, most of the species from Victoria show closer affinities to themselves than they do to Tasmanian species. For instance the OTU group **4-26** is clearly homogeneous, as is the group **39-50** (with the probable inclusion of **35-38** and **27-31** into these two groups at a slightly higher level). The exceptions to this are manyfold:

i) species **1-2** and **3** appear to be more closely related to each other (albeit distantly) than they are to any other species, but beyond this their relationships are obscure,

ii) **52** and **53** (and probably **54**) are interpreted as for i) above,

iii) the species **55-56**, **57** and **58-62** are vastly different from not only each other but also from all other species in the Study.

From this electrophoretic examination of *Engaeus*, therefore, we have the following phyletic groups which show closer affinities within than they do without:

1-3

4-26

39-50

(when the above two cluster they bring in **27-31** and **35-38**)

32-34 (inconclusive relationships)

52-53 (plus **54** possibly)

55-56

57

58-62

72-76, 78-79

(**71** and **51**, **77** and **84** are possibly included in the above group)

80-82

83

It is clear from this preliminary investigation into the phyletic relationships that further information will have to be drawn upon to solve the relationships at higher levels.

SUMMARY

From a total of 80 OTUs, at least 26 distinct species have been delineated. Strong evidence has been provided from sites of both sympatry and allopatry to suggest some further delineations but these must await additional discriminatory information. OTUs in sympatry were analysed on 10 separate occasions, and for each occasion only fixed differences between the OTUs were found, indicating an absence of interbreeding between each sympatric pair. The species boundaries of allopatric OTUs were delineated by taking into account the level of genetic difference between sympatric species and species in the literature, and by examining the geographical pattern of variation of closely related OTUs.

The results of this analysis have confirmed a low level of heterozygosity for OTUs in the decapod genus *Engaeus*; the occurrence of bottlenecks in populations, as predicted by the frequent isolation of populations of species within this genus, was suggested as a possible cause for the low levels.

Some species have shown variation of allelic frequencies over their geographical range and in some cases this variation could be interpreted as showing clinal properties.

Finally, the electrophoretic data has been interpreted to produce some information regarding the phylogenetic affinities of delineated species and other species groups, and this will be assimilated in Chapter 3.

Section 2.3

MULTIVARIATE ANALYSIS OF *ENGAEUS* MORPHOLOGY

Introduction

The applicability and advantages of the multivariate analysis of morphometric data in taxonomic studies have been briefly outlined in Chapter 1.

In general, analyses of morphometric data in the study of parastacid freshwater crayfish have been univariate or bivariate in nature (for instance Francois, 1962; Hopkins, 1970; Suter, 1975). More recently, Sumner (1978) and Morgan (1983) have conducted multivariate analyses of morphometric data from the genera *Parastacoides* and *Euastacus* respectively, by incorporating individuals into the study without the a priori assessment of individuals into species or OTU groups. Such a method has the aim of objectively grouping the individuals according to their morphological similarity, and Morgan in particular gained clear separations of individuals into groups.

In this study, the approach has been to select the OTUs which were analysed in the electrophoretic section, in order to substantiate or clarify the groupings which were found there. This requires the a priori assignment of individuals to groups.

In order to account for the large amount of morphological variability which can be found among individuals in the genus *Engaeus*, it was envisaged that a large number of measurements should be performed on individual crayfish, and in order to cope with more than one variable, multivariate techniques would have to be used.

The above conditions were compatible with the use of the multivariate technique of canonical variate analysis; this technique allows for the a priori assignment of individuals to groups, and has the advantage of measuring the distances between the groups whilst taking into account the variability within each group.

AIM

The aim of this section was to answer the three following questions:

i) What is the level of separation between OTUs which have already been discriminated from other OTUs by the technique of electrophoresis?

ii) What is the level of separation between OTUs which are in doubt, or have not been separated from other OTUs in the electrophoretic study?

iii) Are Tasmanian OTUs morphometrically more similar to each other than they are to the Victorian OTUs?

Materials and Methods

SELECTION OF OTUs

The method for the collection of individuals and the criteria for the selection of OTUs have been outlined in Section 2.1. For this study, and in contrast to the electrophoretic analysis, each OTU was divided into two separate components, males (OTU-**a**) and females (OTU-**b**), and these will each be analysed as discrete units. Thus 92 groups were examined in the morphometric analyses, comprising a male group and a female group for each of 46 OTUs (see Table 1).

OTUs were considered to be suitable for the morphometric analysis if they were represented by a large number of adult specimens (equal to or in excess of approximately 5 males and 5 females). Usually, the OTUs came from the same site as the specimens which were used for the electrophoretic analyses, and in these cases the OTU code was the same as that given to them in the electrophoretic analysis. However, when such large collections of specimens could not be obtained from the electrophoresis sites, specimens were collected from nearby sites where the morphological form was not discernibly different; on these occasions the electrophoretic OTU site code was suffixed with '**A**' (for example **14A**).

There are three exceptions to the methods outlined above.

Firstly, reproductive females at site V48 (OTU **57**) could not be distinguished from intersexed specimens (IS) which are always assumed to be male. For this OTU, the sample (n = 18) was divided into two groups but these groups did not indicate sexual differences.

Secondly, a total of 32 individuals were collected from the site of OTU **28**. This sample was divided into two OTUs or four groups, namely males and females from **28A** and **28B**; this duplication of identical OTUs provided an intrinsic control for the analysis.

On one occasion, a large sample size of specimens was collected from a site which had not been included in the electrophoretic analyses, but the specimens had shown enough variation in their morphological form when compared to their closest morphological neighbour to warrant their inclusion as a separate OTU (**20A**).

In contrast to the electrophoretic studies, these analyses were not split into Victorian and Tasmanian components. The OTU codes, their respective sex, sample size, site number, locality of collection and size range of the individuals used for each OTU are given in Table 1. Figure 1 depicts the locality of these OTUs.

Only adult crayfish were used in this analysis (see size ranges given in Table 1); each individual was preserved in 75% alcohol and 5% glycerol prior to analysis.

METHOD OF ANALYSIS

Selection of Variables

On each preserved individual 55 measurements were performed. The characters selected for measurement were mostly those regarded by previous authors as being of descriptive value in parastacid taxonomy; the list of these characters is given in Table 2.

TABLE 1: The OTU code, site number, locality of collection and sample size for each OTU in the morphometric analysis. In addition the size range (of OCLs or orbital carapace lengths) of the specimens have been included; where the female sample consisted of both reproductively-active and non-reproductive females, the size range of the former have been presented first, followed by the size range of the latter ("NR").

OTU	Sex	n	Site No.	Site Locality	Size Range (OCL, mm)
1-a	IS	13	V07	1.9 km east of Narracan, South Gippsland, Victoria.	16.5 - 26.1
1-b	ISF	9	V07	1.9 km east of Narracan, South Gippsland, Victoria.	20.2 - 25.0
3-a	IS	10	V47B	Ryton Junction, South Gippsland, Victoria.	18.2 - 23.9
3-b	ISF	10	V47B	Ryton Junction, South Gippsland, Victoria.	21.0 - 29.8
4-a	IS	9	V04	Turtons Pass, Otway Ranges, Victoria.	18.6 - 26.4
4-b	ISF	10	V04	Turtons Pass, Otway Ranges, Victoria.	19.0 - 27.3
9-a	IS	11	V63Q	Labertouche Creek near Warragul, Victoria.	16.4 - 24.8
9-b	ISF	9	V63Q	Labertouche Creek near Warragul, Victoria.	16.6 - 28.2
14A-a	IS	10	T03	Little Waterhouse Lagoon, north-eastern Tasmania.	17.5 - 26.3
14A-b	ISF	10	T03	Little Waterhouse Lagoon, north-eastern Tasmania.	17.3 - 24.5
16-a	IS	10	V80	Lilly Pilly Gully, Wilsons Promontory, Victoria.	18.0 - 26.0
16-b	ISF	9	V80	Lilly Pilly Gully, Wilsons Promontory, Victoria.	18.6 - 23.5
18-a	IS	10	V40	Mirboo North, South Gippsland, Victoria.	18.3 - 25.0
18-b	ISF	10	V40	Mirboo North, South Gippsland, Victoria.	17.3 - 24.1
20-a	IS	11	V55	Near South Buchan, East Gippsland, Victoria.	17.1 - 23.9
20-b	ISF	10	V55	Near South Buchan, East Gippsland, Victoria.	17.9 - 23.3
20A-a	IS	9	V74	Betka River, near Genoa, East Gippsland, Victoria.	14.4 - 20.8
20A-b	ISF	6	V74	Betka River, near Genoa, East Gippsland, Victoria.	14.3 - 20.9
23-a	IS	10	V11	Near Mt. Moriac, west of Geelong, Victoria.	20.3 - 27.0
23-b	ISF	10	V11	Near Mt. Moriac, west of Geelong, Victoria.	20.7 - 28.9
24-a	IS	10	V22B	Lake Mumblin, south of Terang, western Victoria.	20.7 - 25.3
24-b	ISF	10	V22B	Lake Mumblin, south of Terang, western Victoria.	19.8 - 24.9
27A-a	IS	9	V72	Between East Warburton and Camberville, Victoria.	22.0 - 36.8
27A-b	ISF	8	V72	Between East Warburton and Camberville, Victoria.	22.8 - 35.8, 20.8 - 25.6 (NR)
28A-a	IS	8	V41	Near Childers, South Gippsland, Victoria.	26.5 - 42.0
28A-b	ISF	9	V41	Near Childers, South Gippsland, Victoria.	27.1 - 34.6, 19.4 - 28.0 (NR)
28B-a	IS	6	V41	Near Childers, South Gippsland, Victoria.	21.6 - 34.4
28B-b	ISF	9	V41	Near Childers, South Gippsland, Victoria.	23.6 - 27.9, 17.7 - 33.3 (NR)
31-a	IS	9	V69	Kongwak, near Jumbunna, South Gippsland, Victoria.	20.1 - 29.6
31-b	ISF	10	V69	Kongwak, near Jumbunna, South Gippsland, Victoria.	25.8 - 31.4
32-a	M	10	V42	Ti-Tree Creek near Bunyip, south-east of Melbourne, Victoria.	10.4 - 19.2
32-b	F	10	V42	Ti-Tree Creek near Bunyip, south-east of Melbourne, Victoria.	14.5 - 18.5, 10.4 - 16.8 (NR)
34-a	M	9	V74	Betka River, near Genoa, East Gippsland, Victoria.	10.6 - 15.1
34-b	F	8	V74	Betka River, near Genoa, East Gippsland, Victoria.	13.0 - 17.6, 10.2 - 14.1 (NR)
35-a	M	10	V51	Tributary of Bufalo River at Dandongadale, Victoria.	13.5 - 24.1
35-b	F	9	V51	Tributary of Bufalo River at Dandongadale, Victoria.	20.7, 12.4 - 21.1 (NR)
38A-a	M	10	C01	Condor Creek, west of Canberra, Australian Capital Territory.	12.4 - 21.7
38A-b	F	10	C01	Condor Creek, west of Canberra, Australian Capital Territory.	16.9, 13.7 - 20.7 (NR)
39-a	M	10	V09	Yarra river Plains at Warburton, Victoria.	19.6 - 29.0
39-b	F	10	V09	Yarra river Plains at Warburton, Victoria.	21.9 - 30.7, 18.1 - 23.5 (NR)
41-a	M	10	V09	Yarra river Plains at Warburton, Victoria.	16.6 - 29.1
41-b	F	10	V09	Yarra river Plains at Warburton, Victoria.	22.7 - 27.7, 17.8 - 27.6 (NR)
43-a	M	7	V71	Kinglake National Park, north of Melbourne, Victoria.	22.8 - 36.2
43-b	F	9	V71	Kinglake National Park, north of Melbourne, Victoria.	24.9 - 39.6, 20.2 - 30.1 (NR)
45-a	M	3	V05	Near Olinda, Dandenong Ranges, Victoria.	18.8 - 30.1
45-b	F	10	V05	Near Olinda, Dandenong Ranges, Victoria.	27.7 - 32.8, 24.3 - 29.3 (NR)
47-a	M	10	V03	Sherbrooke Forest, Dandenong Ranges, Victoria.	19.3 - 28.3
47-b	F	20	V03	Sherbrooke Forest, Dandenong Ranges, Victoria.	23.2 - 30.6, 21.9 - 33.6 (NR)
48-a	M	10	V06	Near Powelltown, east of Melbourne, Victoria.	14.5 - 20.4
48-b	F	11	V06	Near Powelltown, east of Melbourne, Victoria.	14.9 - 20.1, 15.5 - 18.6 (NR)
50-a	M	10	V03	Sherbrooke Forest, Dandenong Ranges, Victoria.	14.1 - 18.3
50-b	F	9	V03	Sherbrooke Forest, Dandenong Ranges, Victoria.	15.5 - 20.2
51-a	M	10	T01	Lilydale, north-eastern Tasmania.	19.8 - 31.1
51-b	F	8	T01	Lilydale, north-eastern Tasmania.	26.4 - 33.4, 21.2 - 23.7 (NR)
52-a	M	10	V66	Lind National Park, East Gippsland, Victoria.	16.0 - 23.7
52-b	F	10	V66	Lind National Park, East Gippsland, Victoria.	16.7 - 22.7, 15.7 - 20.1 (NR)
53-a	M+IS	8	V63S	Labertouche Creek near Warragul, Victoria.	14.1 - 20.3
53-b	F	4	V63S	Labertouche Creek near Warragul, Victoria.	16.0 - 19.0, 15.7 (NR)
54-a	IS	4	V68	Near Mallacoota, East Gippsalnd, Victoria.	14.9 - 17.8
54-b	ISF	4	V68	Near Mallacoota, East Gippsalnd, Victoria.	17.6 - 20.4
56-a	M+IS	7	V25	Glenelg River at Dartmoor, western Victoria.	15.4 - 24.3
56-b	F	9	V25	Glenelg River at Dartmoor, western Victoria.	20.1 - 26.0, 17.5 - 24.0 (NR)
57-a	IS	10	V48	Lilly Pilly Gully, Wilsons Promontory, Victoria.	16.4 - 24.7
57-b	IS	8	V48	Lilly Pilly Gully, Wilsons Promontory, Victoria.	14.3 - 20.0
58-a	M	10	V02	Near Gisborne, north of Melbourne, Victoria.	13.9 - 28.7
58-b	F	10	V02	Near Gisborne, north of Melbourne, Victoria.	23.9, 12.6 - 28.3 (NR)
60-a	M	10	V31	Near Halls Gap, Grampian Ranges, western Victoria.	16.9 - 24.7
60-b	F	11	V31	Near Halls Gap, Grampian Ranges, western Victoria.	23.5 - 32.4, 19.3 - 23.0 (NR)
71-a	M	9	T48	Rocky Cape National Park, north-western Tasmania.	11.0 - 16.1
71-b	F	10	T48	Rocky Cape National Park, north-western Tasmania.	10.8 - 17.4
73-a	M	10	T32	Browns Creek near Port Sorell, northern Tasmania.	15.1 - 25.5
73-b	F	10	T32	Browns Creek near Port Sorell, northern Tasmania.	19.2 - 29.6, 17.4 - 19.6 (NR)
74-a	M	10	T40A	Surveyors Creek north of Scottsdale, north-eastern Tasmania.	15.5 - 23.8
74-b	F	10	T40A	Surveyors Creek north of Scottsdale, north-eastern Tasmania.	18.1 - 24.4
75A-a	M	10	T02	Creek at Lefroy, near George Town, northern Tasmania.	13.1 - 21.4
75A-b	F	10	T02	Creek at Lefroy, near George Town, northern Tasmania.	15.3 - 24.1, 11.0 - 16.1 (NR)
76A-a	M	5	T18	Swamp at Birralee, north of Westbury, northern Tasmania.	16.6 - 27.6
76A-b	F	5	T18	Swamp at Birralee, north of Westbury, northern Tasmania.	15.1 - 24.8
77-a	IS	10	T26Q	Mt. Strzelecki, Flinders Island, Bass Strait.	15.6 - 25.1
77-b	ISF	10	T26Q	Mt. Strzelecki, Flinders Island, Bass Strait.	18.8 - 25.3
78-a	M+IS	8	T15A	Tributary of Dip River, north-western Tasmania.	17.8 - 23.2
78-b	F	10	T15A	Tributary of Dip River, north-western Tasmania.	18.7 - 25.3, 16.3 - 22.0 (NR)
78A-a	M	9	T14	Swamp on Table Cape, near Wynyard, northern Tasmania.	17.5 - 29.5
78A-b	F	9	T14	Swamp on Table Cape, near Wynyard, northern Tasmania.	20.9 - 26.7, 15.0 - 21.0 (NR)
81-a	M+IS	12	T15B	Tributary of Dip River, north-western Tasmania.	17.3 - 28.7
81-b	F	8	T15B	Tributary of Dip River, north-western Tasmania.	25.6 - 30.5, 18.2 - 19.0 (NR)
82-a	M	10	T20	Weetah, near Elizabeth Town, northern Tasmania.	15.6 - 24.9
82-b	F	10	T20	Weetah, near Elizabeth Town, northern Tasmania.	20.0 - 24.6, 16.1 - 21.0 (NR)
83-a	IS	9	T04B	Pearly Brook, north of Scottsdale, north-eastern Tasmania.	13.4 - 22.7
83-b	ISF	10	T04B	Pearly Brook, north of Scottsdale, north-eastern Tasmania.	15.0 - 22.9
84-a	M	10	T05	Bradshaws Creek near Herrick, north-eastern Tasmania.	18.5 - 32.7
84-b	F	10	T05	Bradshaws Creek near Herrick, north-eastern Tasmania.	19.4 - 23.8 (NR)

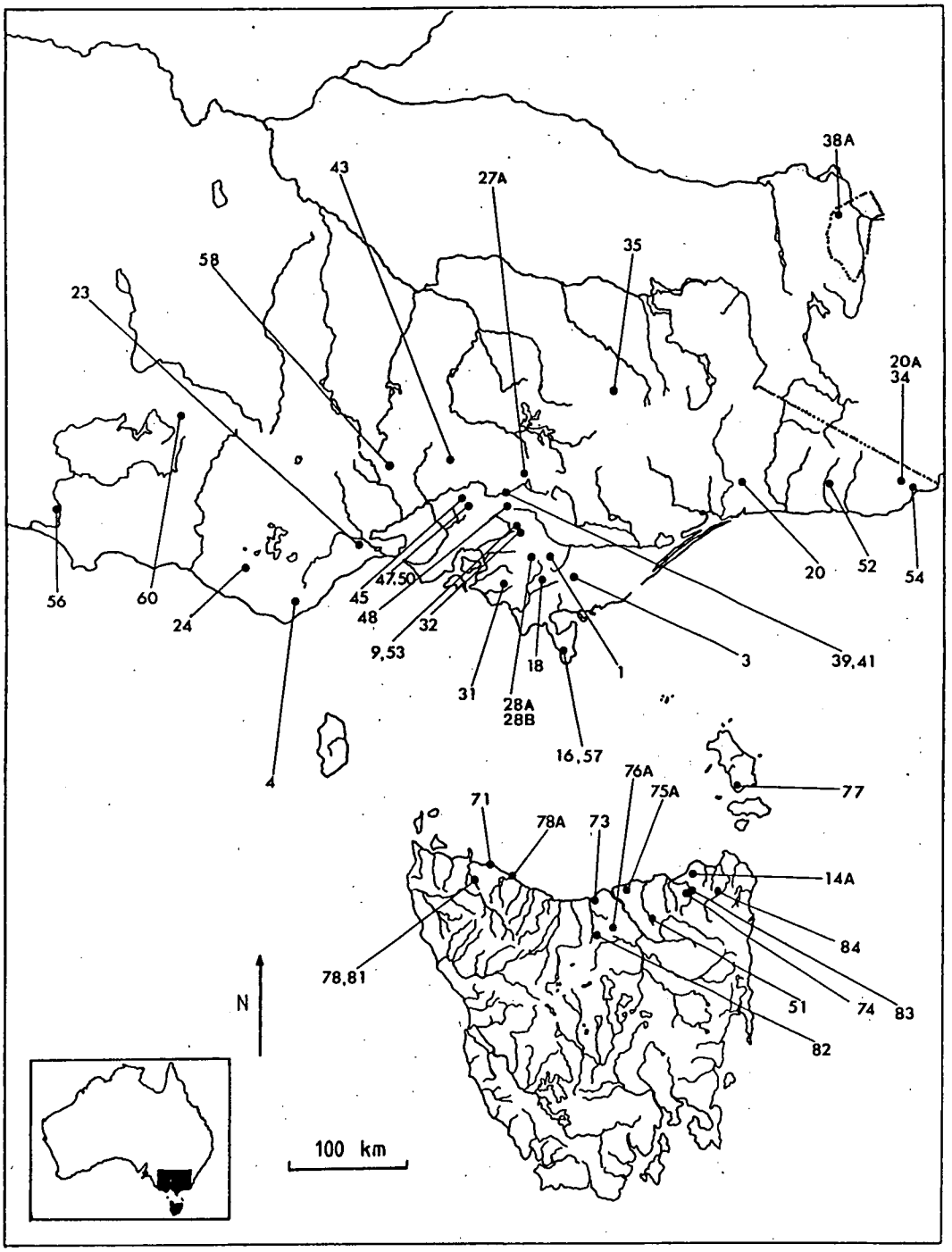


FIGURE 1: Map of Victoria and Tasmania showing the location of each OTU used in the multivariate analysis.

ABBR.	MEASUREMENT	VARIABLE STATUS
CEPHALIC REGION		
RL	ROSTRAL LENGTH	D
RW	ROSTRAL WIDTH	D
EW	EYE WIDTH	E
OW	ORBITAL WIDTH	OK
ASL	ANTENNAL SCALE LENGTH	D
ASW	ANTENNAL SCALE WIDTH	D
IATL	LENGTH OF INNER FLAGELLUM OF ANTENNULE	A
OATL	LENGTH OF OUTER FLAGELLUM OF ANTENNULE	A
ATL	LENGTH OF ANTENNAL FLAGELLA	A
3MXL	LENGTH OF EXOPODITE OF THIRD MAXILLIPED	A
3MNL	LENGTH OF ISCHIUM OF THIRD MAXILLIPED	D
PLEOPODS		
1PBL	LENGTH OF BASE OF FIRST PLEOPOD	B
1PBW	WIDTH OF BASE OF FIRST PLEOPOD	OK
1PXL	LENGTH OF EXOPODITE OF FIRST PLEOPOD	B
1PNL	LENGTH OF ENDOPODITE OF FIRST PLEOPOD	B
2PBL	LENGTH OF BASE OF SECOND PLEOPOD	B
2PBW	WIDTH OF BASE OF SECOND PLEOPOD	B
2PXL	LENGTH OF EXOPODITE OF SECOND PLEOPOD	B
2PNL	LENGTH OF ENDOPODITE OF SECOND PLEOPOD	B
THIRD PEREIOPOD (SECOND WALKING LEG)		
3PDL	LENGTH OF DACTYL	E
3PPL	LENGTH OF PROPODUS	E
3PMW	WIDTH OF MERUS	D
3PML	LENGTH OF MERUS	D
FIFTH PEREIOPOD (FOURTH WALKING LEG)		
5PDL	LENGTH OF DACTYL	OK
5PPL	LENGTH OF PROPODUS	OK
5PMW	WIDTH OF MERUS	D
5PML	LENGTH OF MERUS	D

ABBR.	MEASUREMENT	VARIABLE STATUS
CEPHALOTHORAX		
CL	CEPHALIC LENGTH	E
TL	THORACIC LENGTH (AREOLAR LENGTH)	E
CW	CARAPACE WIDTH	E
CD	CARAPACE DEPTH	D
AW	AREOLAR WIDTH	OK
ABDOMEN		
ABSL	TOTAL LENGTH OF ABDOMINAL SOMITES	A
1ASW	WIDTH OF FIRST ABDOMINAL SOMITE	D
2ASW	WIDTH OF SECOND ABDOMINAL SOMITE	B
3ASW	WIDTH OF THIRD ABDOMINAL SOMITE	B
4ASW	WIDTH OF FOURTH ABDOMINAL SOMITE	B
5ASW	WIDTH OF FIFTH ABDOMINAL SOMITE	B
6ASW	WIDTH OF SIXTH ABDOMINAL SOMITE	B
TAIL FAN		
TW	TELSON WIDTH	B
TL	TELSON LENGTH	B
UEXL	LENGTH OF OUTER RAMUS OF UROPOD	B
UEXW	WIDTH OF OUTER RAMUS OF UROPOD	B
UENL	LENGTH OF INNER RAMUS OF UROPOD	B
UENW	WIDTH OF INNER RAMUS OF UROPOD	B
CHELAE		
CDL	DACTYL LENGTH	C
CDW	DACTYL WIDTH	C
CPL	PROPODUS LENGTH	C
CPW	PROPODUS WIDTH	C
CPD	PROPODUS DEPTH	C
CCL	CARPUS LENGTH	C
CCW	CARPUS WIDTH	C
CML	MERUS LENGTH	C
CMW	MERUS WIDTH	C
CIW	ISCHIUM WIDTH	C

TABLE 2: Showing the measurements performed on each individual for the morphometric analyses, giving their abbreviation (ABBR.) and their variable status (where A = measurements which were inconsistently expressed, B = measurements which were dimorphic for females, C = measurements of chelae, D = non-normal distributions, E = duplication of information and OK = satisfactory for multivariate analysis: see text).

Measurements were performed using vernier callipers or a graticule attached to the lens of a binocular microscope and recorded to an accuracy of $^{+}/_{-}$ 0.1 mm. No meristic characters were incorporated in the analysis.

Except for the chelae, all measurements were performed on the right side of the specimen unless the appendage was broken or missing, in which case the left appendage was measured; for the chelae, both right and left appendages were measured on each individual. Most of the measurements are diagrammatically presented in Chapter 5.

Each measurement was required to conform to a set of requirements before it was included in the multivariate analysis. Measurements which were inconsistently expressed from species to species (for instance the inner flagellum of the antennule, which can be absent on all individuals of some species) or from individual to individual (for instance the outer flagella of the antennule, which can both be broken on some specimens) were excluded from the analysis (these characters were given the variable status 'A' in Table 2).

Each female OTU potentially contained a different ratio of reproductively-active and non-reproductive females (although all individuals were adults; see Glossary, Chapter 5). This means that any variable which is significantly different between these two forms of female produces values from a heterogeneous 'population', and therefore should be excluded from the analysis. Horwitz (1987, in press; Appendix 1) described some significant changes in female crayfish morphology from the non-reproductive to the reproductive form in two species of *Engaeus*; accordingly, all tail fan measurements, most abdominal somite widths and most pleopodal measurements were excluded from the analysis (variable status 'B' in Table 2).

Individuals of some species within the genus *Engaeus* frequently exhibit heterochelosity, or altered proportions of one chela compared to the other (see Chapter 5). This dimorphism affected all measurements of the chelae and since there was no way of consistently eliminating all of the within-OTU variation in chelae, they were all excluded from the analysis (variable status 'C' in Table 2).

The filtering process thus far eliminated 33 measurements; each of the remaining 22 measurements was examined for the statistical attributes which are required for the multivariate analyses. Each measurement was standardized for size using the following conversion:

$$V_{sum} = \Sigma V = V_1 + V_2 + V_3 + \ldots\ldots + V_{22}$$

and

$$Z_i = \log (V_i + 2 / V_{sum})$$

where V = the raw value for each respective measurement, V_i is the raw value of the ith measurement, and Z_i is the value of the ith measurement which has been standardized for size. All variables (Z's) were then tested for errors in the data set, for normality and a one-way

analysis of variance was performed, all by using the statistical program TEDDYBEAR (adapted for use at the University of Tasmania from Technical Report T5 of the University of Otago Computing Centre, Dunedin, New Zealand).

Eleven variables were rejected from further analysis since they failed to produce a straight line on the normal probability distribution plot (as described by Hogben et al., 1971) and were thus assumed to be non-normally distributed. These variables were given variable status 'D' in Table 2.

Of the remaining variables, 5 were eliminated because they gave identical OTU separations in the analysis of variance when compared to the results of another variable and thus only served to duplicate the available information (for instance 3PPL and 3PDL gave the same information as 5PPL and 5PDL respectively; the former variables were eliminated and given status 'E' in Table 2).

Thus, 6 variables were chosen for use in the multivariate analysis; OW, 1PBW, 5PDL, 5PPL, CL and AW. The ranges of Z's for each OTU and for each variable are given in Table 3. The specific details of the normality tests and the analysis of variance for each variable are given in Table 4. It is noticeable from these that on 3 out of 6 occasions the correlation between the residual mean square and the mean were significant. However since the probability plots were normal and since, due to the large number of groups, even small alterations in the within-groups variances could produce an overall significant variance (G. McPherson, Department of Mathematics, Univerity of Tasmania, pers. comm.), each of the six variable was considered to be suitable for further analysis.

The linear sequence of univariate separations produced from the analysis of variance using Duncan's Multiple Range Test are given for each variable in Figure 2 (for OTU sites only since the factor of sex was not analysed).

Finally, canonical variate analysis can only be meaningfully applied if the interrelationships between the variables are consistently expressed for all the groups being investigated. To test for this relationship a correlation matrix was produced from the means for each variable for each group; the results of this analysis showed that all six variables were significantly correlated with each other (Table 5), and as such a consistent interrelationship between the variables was assumed.

In summary, therefore, from a total of 55 different measurements performed on each individual crayfish, 6 were chosen since they conformed to the necessary biological and statistical requirements, and these 6 variables were included in the canonical variate analysis.

Canonical Variate Analysis

The mathematical derivation of canonical variate analysis is given in Seal (1964) and Blackith and Reyment (1971). The canonical variate analysis for this work was performed on the statistical program GENSTAT (Alvey et al., 1977) and Mr. G. McPherson of the Mathematics Department, University of Tasmania was consulted on the particulars of its use.

Essentially, canonical variate analysis incorporates variables simultaneously and in doing so utilizes the interrelationships between the variables. The basic strategy of

	OW	1PBW	5PDL	5PPL	CL	AW
1-a	1894-2323	507-664	1476-1802	2177-2698	2996-4039	1452-1949
1-b	1950-2164	499-604	1424-1576	2261-2456	3102-3489	1562-1752
3-a	1874-2187	577-730	1420-1600	2314-2688	3271-3898	1463-1744
3-b	1711-2027	547-634	1267-1545	2058-2469	2761-3528	1354-1616
4-a	1794-2209	574-679	1286-1547	2081-2615	2869-3489	1457-1724
4-b	1550-2143	419-680	1116-1546	1829-2493	2771-3493	1234-1711
9-a	1968-2392	604-753	1347-1669	2119-2643	2954-3814	1460-1823
9-b	1844-2316	630-713	1237-1717	2006-2559	2691-3719	1347-1765
14A-a	1863-2279	549-692	1318-1605	2032-2537	2831-3635	1463-1887
14A-b	1837-2270	604-680	1361-1683	2124-2569	2942-3632	1542-1813
16-a	1840-2216	568-686	1316-1606	2114-2539	2811-3590	1455-1809
16-b	1964-2280	611-727	1425-1733	2206-2607	2985-3622	1580-1773
18-a	1899-2244	548-723	1288-1616	2162-2585	2976-3714	1430-1711
18-b	1856-2214	600-714	1392-1567	2222-2672	3067-3776	1410-1670
20-a	1869-2399	612-739	1309-1608	2224-2602	3012-3813	1496-1727
20-b	1989-2263	646-707	1378-1557	2246-2533	3029-3645	1409-1701
20A-a	2127-2614	557-742	1077-1484	1864-2482	2400-3366	1131-1381
20A-b	2132-2558	571-693	1430-1720	2460-2949	3336-4290	1547-1796
23-a	1841-2183	599-724	1350-1564	2013-2336	2825-3406	1374-1567
23-b	1775-2130	611-727	1264-1513	1939-2333	2631-3331	1301-1601
24-a	1839-2183	536-652	1311-1467	2110-2373	2933-3345	1556-1346
24-b	1964-2155	562-675	1326-1487	2190-2477	2960-3416	1375-1681
27A-a	1546-2005	558-699	1089-1490	1813-2416	2332-3265	1072-1385
27A-b	1558-2078	557-742	1077-1484	1864-2482	2400-3366	1131-1381
28A-a	1436-1884	618-714	997-1282	1624-2154	2149-2935	1020-1380
28A-b	1554-2149	563-737	1114-1571	1852-2489	2428-3573	1033-1380
28B-a	1536-2040	614-709	1103-1349	1822-2411	2433-3372	1135-1392
28B-b	1623-2286	570-784	1140-1573	1894-2671	2519-3695	1160-1411
31-a	1740-2143	632-735	1197-1477	2050-2527	2741-3625	1245-1563
31-b	1699-1892	571-698	1163-1330	1972-2247	2695-3142	1139-1392
32-a	2004-2567	670-836	1552-2110	2267-3036	2968-4156	1812-2583
32-b	1977-2617	636-854	1632-2168	2316-3071	3121-4156	1906-2514
34-a	2392-2706	714-876	1712-1979	2578-3100	3692-4464	2103-2448
34-b	2258-2813	619-857	1593-1955	2543-3187	3834-4662	1998-2601
35-a	1785-2395	562-683	1231-1827	2239-3189	2866-4180	1465-1752
35-b	1904-2457	594-692	1363-1801	2483-3352	3229-4403	1292-1793
38-a	1885-2553	502-640	1320-1822	2438-3328	3180-4346	1360-1760
38-b	2069-2276	470-615	1443-1747	2533-3182	3238-4135	1344-1712
39-a	1698-2061	572-656	1198-1507	1985-2451	2735-3466	1428-1630
39-b	1662-2114	570-757	1118-1469	1930-2556	2614-3614	1369-1703
41-a	1709-2201	590-663	1099-1523	1971-2667	2740-3944	1425-1842
41-b	1748-2160	563-685	1144-1501	2072-2612	2790-3682	1441-1826

TABLE 3: The ranges of Z-values (X 1000; see text) for each OTU and for each of the six variables used in the canonical variate analysis (see Table 2 for the abbreviations of each variable).

	OW	1PBW	5PDL	5PPL	CL	AW
43-a	1537-1928	528-630	1078-1360	1772-2276	2330-3206	1246-1614
43-b	1464-2083	523-690	1012-1440	1696-2499	2148-3421	1281-1741
45-a	1753-2213	564-649	1298-1616	1935-2559	2791-3845	1173-1450
45-b	1652-2069	503-721	1207-1481	1851-2280	2628-3304	1092-1337
47-a	1779-2140	626-691	1269-1465	1988-2425	2779-3697	1191-1657
47-b	1710-2073	504-726	1136-1398	1929-2301	2684-3420	1218-1464
48-a	2044-2396	551-861	1433-1750	2412-2873	2536-4295	1481-1859
48-b	1993-2423	561-736	1407-1710	2378-2884	3576-4285	1546-1803
50-a	2261-2524	588-727	1577-1712	2498-2932	3730-4488	1712-1905
50-b	2165-2474	544-711	1463-1676	2486-2828	3605-4234	1621-1895
51-a	1629-2120	558-621	1169-1532	1999-2619	2534-3399	1406-1732
51-b	1580-2069	582-660	1126-1443	1955-2533	2417-3062	1337-1720
52-a	1929-2376	548-719	1294-1734	2256-2863	3020-3920	1481-1843
52-b	2016-2371	565-682	1337-1730	2316-2895	3150-3984	1504-1846
53-a	2268-2601	552-754	1465-1822	2370-2711	3560-4433	1723-2047
53-b	2286-2547	694-732	1537-1725	2435-2755	3731-4210	1671-1922
54-a	2278-2520	708-839	1640-1733	2624-2877	3733-4182	1839-1975
54-b	2120-2355	699-761	1447-1567	2486-2639	3486-3744	1756-1881
56-a	1991-2534	635-859	1386-1861	2187-2814	3195-4350	1598-1747
56-b	1907-2411	556-673	1398-1570	2063-2771	2973-3922	1449-1708
57-a	1524-2302	551-662	1325-1620	2236-2839	3145-3998	1694-2044
57-b	1846-2540	554-706	1495-1700	2493-3046	3566-4528	1882-2133
58-a	1666-2435	593-842	1249-1709	1988-2915	2498-3970	1395-1777
58-b	1660-2455	688-766	1312-1759	1955-3050	2522-4152	1458-1864
60-a	1786-2158	686-785	1330-1674	2210-2651	2805-3561	1302-1570
60-b	1488-2027	628-831	1213-1619	1873-2561	2663-3232	1081-1543
71-a	2496-2953	654-870	1772-2106	2720-3293	3911-4939	2021-2480
71-b	2331-3079	695-822	1681-2104	2560-3272	3680-4938	2132-2388
73-a	1890-2483	577-813	1412-1781	2167-2795	2879-4052	1698-2378
73-b	1750-2345	577-786	1269-1691	1966-2602	2676-3702	1626-2145
74-a	1944-2375	560-683	1343-1792	2264-2808	3023-3990	1601-1850
74-b	1939-2270	518-610	1385-1637	2223-2617	3047-3632	1553-1771
75A-a	2054-2597	593-674	1432-1859	2361-2940	3302-4276	1825-2437
75A-b	1907-2859	620-786	1378-1946	2167-3180	2938-4859	1739-2488
76A-a	1837-2259	543-775	1234-1652	1887-2591	2702-3565	1576-2024
76A-b	1802-2301	652-741	1335-1785	2136-2660	2858-3678	1654-2064
77-a	2058-2614	511-610	1531-1908	2398-3022	3101-4285	1537-1958
77-b	2001-2358	568-611	1435-1726	2294-2754	3119-3771	1582-1812
78-a	2051-2318	555-730	1479-1672	2376-2702	3247-3832	1851-2056
78-b	1894-2494	577-729	1363-1740	2303-2844	3108-4005	1739-2138
78A-a	1783-2453	611-729	1271-1643	1944-2681	2848-3927	1641-2139
78A-b	1877-2682	628-775	1339-1810	2135-2757	2921-4149	1654-2202
81-a	1695-2278	548-644	1198-1634	2111-2844	2720-3931	1533-1932
81-b	1662-2187	524-671	1141-1544	2069-2735	2733-3750	1544-1920
82-a	1829-2265	638-759	1192-1653	2282-2981	3073-3928	1809-2177
82-b	1835-2259	577-684	1195-1618	2316-2945	3057-3920	1804-2174
83-a	2082-2601	520-669	1407-1822	2328-3077	3263-4558	1353-1766
83-b	2035-2648	469-714	1346-1700	2363-2967	3209-4201	1309-1816
84-a	1577-2168	518-608	1191-1607	1957-2659	2497-3553	1228-1652
84-b	1945-2119	527-608	1441-1649	2311-2668	3097-3543	1441-1632

TABLE 3 (cont.): The ranges of Z-values (X 1000; see text) for each OTU and for each of the six variables used in the canonical variate analysis (see Table 2 for the abbreviations of each variable).

VARIABLE 1 - ORBITAL WIDTH

Tests for normality:

I. Probability plot straight.

II. Means vs. Variances R = 0.128979, DF = 90, p = 0.22045, NS.

Grand mean = 0.020944. Coefficient of Variation = 7.48%.

ANALYSIS OF VARIANCE

	SS	DF	MS	F	P
OTUs	0.0359816	45	0.00007996	32.5396	0.000000
SEX	0.0000137	1	0.00001376	5.5647	0.018577
O X S	0.0002080	45	0.00000462	1.8808	0.000550
Error	0.0018776	764	0.00000246		
Total	0.0057986	855	0.00000678		

VARIABLE 2 - BASE WIDTH OF 1ST PLEOPOD

Tests for normality:

I. Probability plot straight.

II. Means vs. Variances R = 0.1675542, DF = 90, p = 0.110413, NS.

Grand mean = 0.0064285. Coefficient of Variation = 7.37%.

ANALYSIS OF VARIANCE

	SS	DF	MS	F	P
OTUs	0.0002145	45	0.00000477	21.2080	0.000000
SEX	0.0000002	1	0.00000023	1.0385	0.308496
O X S	0.0000183	45	0.00000040	1.8113	0.001123
Error	0.0001717	764	0.00000022		
Total	0.0004058	855	0.00000047		

VARIABLE 3 - DACTYL LENGTH OF 4TH PEREIOPOD

Tests for normality:

I. Probability plot straight.

II. Means vs. Variances R = 0.209599, DF = 90, p = 0.044937, p < 0.05.

Grand mean = 0.014925. Coefficient of Variation = 7.82%.

ANALYSIS OF VARIANCE

	SS	DF	MS	F	P
OTUs	0.0019803	45	0.00004401	32.2656	0.000000
SEX	0.0000080	1	0.00000798	5.8547	0.015768
O X S	0.0001427	45	0.00000317	2.3260	0.000004
Error	0.0010420	764	0.00000136		
Total	0.0032131	855	0.00000376		

VARIABLE 4 - PROPODAL LENGTH OF 5TH PEREIOPOD

Tests for normality:

I. Probability plot straight.

II. Means vs. Variances R = 0.288597, DF = 90, p = 0.005272, p < 0.01.

Grand mean = 0.02445. Coefficient of Variation = 7.82%.

ANALYSIS OF VARIANCE

	SS	DF	MS	F	P
OTUs	0.0043461	45	0.00009658	26.4162	0.000000
SEX	0.0000093	1	0.00000932	2.5490	0.110779
O X S	0.0003521	45	0.00000782	2.1398	0.000032
Error	0.0027933	764	0.00000366		
Total	0.0076370	855	0.00000893		

VARIABLE 5 - CEPHALIC LENGTH

Tests for normality:

I. Probability plot straight.

II. Means vs. Variances R = 0.219595, DF = 90, p = 0.035446, p < 0.05.

Grand mean = 0.034003. Coefficient of Variation = 9.14%.

ANALYSIS OF VARIANCE

	SS	DF	MS	F	P
OTUs	0.0112844	45	0.00025077	25.9800	0.000000
SEX	0.0000847	1	0.00008474	8.7792	0.003141
O X S	0.0010077	45	0.00002239	2.3201	0.000004
Error	0.0073743	764	0.00000965		
Total	0.0199828	855	0.00002337		

VARIABLE 6 - AREOLAR WIDTH

Tests for normality:

I. Probability plot straight.

II. Means vs. Variances R = 0.094282, DF = 90, p = 0.37135, NS.

Grand mean = 0.01654. Coefficient of Variation = 8.15%.

ANALYSIS OF VARIANCE

	SS	DF	MS	F	P
OTUs	0.0056244	45	0.00012499	68.7525	0.000000
SEX	0.0000039	1	0.00000392	2.1596	0.142089
O X S	0.0001442	45	0.00000320	1.7630	0.001822
Error	0.0013888	764	0.00000182		
Total	0.0073085	855	0.00000855		

TABLE 4: Some statistical properties for each of the six variables used in the multivariate analysis, showing the tests for normality (probability plots and correlations between the residual mean square and the mean for each OTU) and the one-way analysis of variance (where "O X S" should read "OTUs X SEX").

OW

28A 27A 43 39 51 31 28B 60 45 81 47 41 4 3 23 84 14A 82 24 18 9 57 74 76A 16 58 20 1 52 35 78A 73 56 78 48 38 77 83 54 32 20A 75A 50 53 34 71

1PBW

84 1 38 77 83 81 74 43 51 4 24 45 57 41 39 50 27A 20A 52 47 3 35 14A 78 18 16 31 28A 56 48 75A 28B 82 20 23 9 76A 78A 53 73 60 32 58 54 71 34

5PDL

28A 43 27A 39 31 41 28B 47 51 81 45 24 4 82 23 9 20 14A 84 74 60 52 3 57 18 76A 58 35 83 78A 16 48 73 38 20A 78 1 54 50 56 53 75A 77 34 32 71

5PPL

28A 43 27A 45 23 39 47 31 28B 14A 9 51 41 4 24 60 76A 81 18 16 20 3 84 74 1 78A 73 56 52 78 57 58 53 82 77 48 54 83 75A 50 32 20A 35 34 38 71

CL

28A 27A 43 51 39 60 23 28B 31 4 14A 45 9 24 81 47 84 76A 41 18 16 74 20 3 58 82 52 73 1 78A 78 56 77 35 57 32 83 38 54 20A 75A 48 53 50 34 71

AW

28A 27A 45 28A 31 47 60 23 43 84 24 39 83 4 3 38 9 35 51 18 20 41 56 14A 58 52 76A 16 74 81 20A 48 1 77 50 53 54 37 78 78A 82 73 75A 32 71 34

FIGURE 2: The results of the univariate Duncan's Multiple Range Test for each of the six variables used in the canonical variate analysis. The OTUs are arranged in a linear sequence so that their nearest neighbors on either side exhibit the most similar values for that variable.

	OW	IPBW	5PDL	5PPL	CL	AW
OW		0.53449	0.87161	0.81347	0.81994	0.71449
IPBW	5.999(***)		0.43958	0.22991	0.31400	0.44670
5PDL	16.868(***)	4.6429(***)		0.86465	0.87906	0.81522
5PPL	13.269(***)	2.7661(**)	16.328(***)		0.93845	0.73047
CL	13.588(***)	3.1376(***)	17.494(***)	25.774(***)		0.74085
AW	9.6880(***)	4.7366(***)	13.354(***)	10.147(***)	10.464(***)	

TABLE 5: The correlation matrix for the six variables used in the canonical variate analysis produced from the means for each variable for each group (OTU), showing the correlation coefficient above the diagonal and the Students t-value below the diagonal (with levels of significance for 90 degrees of freedom, where ** = $p < 0.01$ and *** = $p < 0.001$).

canonical variate analysis is to calculate the linear combinations of the variables in order to maximize the ratio of between- to within-group variance. The resulting linear combinations are the "canonical variables". These canonical variables are created in such a way that the first gives the maximal possible separation of the groups, the second gives the next greatest separation of the groups, and so on. The number of canonical variables (k) is equal to either the number of measured variables, or the number of groups less one, whichever is the smaller value; in this case, therefore, k = 6. The canonical variables are represented by "latent roots" ($\emptyset_i$ or "eigenvalues") and the percentage of variation which is explained by each is given as

$$100 \times \emptyset_1 / (\emptyset_1 + \emptyset_2 + \emptyset_3 + \emptyset_4 + \emptyset_5 + \emptyset_6)$$

The usefulness of the latent root can be tested using the statistic *alpha*

$$alpha_1 = \{r - ((p - g + 2)/2)\} \ln \{(1 + \emptyset_1)(1 + \emptyset_2).....(1 + \emptyset_6)\}$$

where r = residual degrees of freedom, p = the number of measured variables, and g = the number of groups or treatments. The *alpha* statistic has a sampling distribution which is approximated by the Chi-square distribution and therefore a level of significance can be appplied to each latent root with its associated percentage variance.

Separation of OTUs

Since each latent root is represented by a canonical variate mean for each OTU or group, then those which contribute a significant amount of variance to the overall picture can then be plotted for a visual assessment of the separation between the groups. This is one way in which the degree of separation between the groups can be ascertained. A quantitative measure can be gained through the use of the Mahalanobis distance (D-value). If this measure for any pair of OTUs exceeds that of the computed statistic D_5

$$D_5 = \sqrt{\{ (1/n_1 + 1/n_2) (r / (r + 1 - P)) P F_{0.05,P,r+1-P} \}}$$

where n_1 and n_2 = the numbers of individuals in the OTUs 1 and 2, r = the residual degrees of freedom, P = the number of variables and $F_{0.05,P,r+1-P}$ = the tabulated F-value from the F-distribution, then the pair are said to be separated by a statistically significant distance.

In this study, any two OTUs **X** and **Y** were considered to be significantly distanced from one another if the males from **X** were significantly different to the males from **Y**, and likewise for the females. If at least these two cells showed non-significant differences, then the OTUs were interpreted as being morphometrically indistinct.

Results

CANONICAL VARIABLES

The latent root, the percentage of variation explained and the level of significance for each canonical variable are given in Table 6; these results show that each of the six canonical variables contributed a significant amount of variation to the overall analysis. Thus to depict the spatial separation between the groups accurately, only a method which plots multi-dimensional data could be used and this was not available. A two- or three-dimensional plot using the 1st, 2nd and 3rd canonical variate means (see Table 7) of the separation between the OTUs would only be misleading since such plots could only explain a maximum of 50 % - 60 % of the overall variation (see Table 6).

MAHALANOBIS DISTANCES

A more informative indication of the separation between the OTUs is given in Table 8 where the Mahalanobis Distances and their levels of significance are presented for each OTU pair. The most important feature in this Table is that on all occasions, for each OTU, the difference between male (OTU-**a**) and female (OTU-**b**) is not significant. In addition the amount of separation between the OTUs **28A** and **28B**, which are merely two samples of the same population, is not significant. Therefore the canonical variate analysis, as expressed by the Mahalanobis Distances, give separations between the OTUs in this study which are biologically relevant. However, the analysis in many cases fails to distinguish between morphologically and electrophoretically distinct groups and many examples of this can be found. For instance the Victorian OTU **4** is inseparable from the OTUs **9, 14A, 16, 18, 20, 23, 24, 39, 43** and **51** in this analysis, whilst in the electrophoretic analysis, OTU **4** is discrete. Other examples of this can be found for the OTUs **52, 53** and **54.**

We can conclude from this that this disciminatory technique does not have the power to distinguish the OTUs. In other words, a consistently significant difference or clear-cut discrimination between any OTU pair can be considered as a valid interpretation of species differences; however a failure to discriminate between OTUs has more doubtful consequences, since it may represent either a true similarity between the OTUs or merely a convergence over the measured variables.

SEPARATION BETWEEN ELECTROPHORETICALLY CLOSE OTUs

The most powerful application of these results therefore lies in the examination of the OTUs which were too close electrophoretically to provide any conclusive evidence for the delineation of species.

OTUs 73 and 75A

The members of this OTU pair are significantly different in two out of the four cells (between **73-b & 75A-a** and **73-b & 75A-b**), but nonsignificantly different in the remainder. Since significant differences are more potent than nonsignificant ones, this provides further evidence to separate the two OTUs and consider them as separate species.

OTUs 9, 14A, 16, 18, 20, 20A, 23 and 24

In general this technique failed to provide evidence towards possible OTU

	1	2	3	4	5	6
LATENT ROOTS	0.8491	0.7586	0.7285	0.5713	0.5344	0.3634
% VARIANCE	22.313	19.936	19.144	15.013	14.044	9.5496
alpha VALUE	2350.70	1855.25	1400.25	959.16	594.93	249.84
SIGNIFICANCE	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$

TABLE 6: The latent root, the percentage variation explained, the *alpha* value and its level of significance for each canonical variable (1-6). The *alpha* value is calculated as described in the Methods.

OTU	1	2	3	4	5	6
1-a	-0.2393	1.0581	0.1366	1.4195	1.0770	0.5962
1-b	-0.1366	0.5898	0.0515	0.8281	0.9668	0.9737
3-a	0.2994	0.1486	0.3153	0.1426	0.4951	-0.9459
3-b	0.3833	0.1503	0.4861	0.0689	0.9720	-0.0743
4-a	0.1661	-0.0007	-0.2968	-0.0079	0.1570	0.5363
4-b	0.1031	-0.0186	-0.3466	-0.2334	0.5744	0.7071
9-a	0.0946	0.6452	-0.4765	-0.1714	-0.1282	-0.1581
9-b	0.0095	0.7431	-0.3242	-0.5156	-0.0881	0.0944
14A-a	-0.2271	0.7695	-0.5257	-0.2553	0.0295	0.8444
14A-b	-0.3500	0.8060	-0.7409	-0.2881	0.2419	0.2950
16-a	-0.4961	0.2910	-0.3742	0.0455	0.3355	0.2607
16-b	-0.3025	0.4612	-0.5270	-0.0286	0.5924	-0.0920
18-a	0.1027	0.3515	-0.4669	0.0385	0.1632	0.0354
18-b	0.1510	0.2143	-0.6233	-0.0330	0.4246	-0.2262
20-a	0.2679	-0.0633	-0.2802	-0.0371	-0.7646	0.0205
20-b	0.2580	0.0007	-0.4894	-0.1823	-0.2679	-0.1995
20A-a	0.6222	-0.2274	-0.1946	1.0719	-0.3702	-0.1091
20A-b	0.5786	-0.5595	0.1939	0.9167	-1.0901	-0.0059
23-a	0.2236	0.9950	-0.5640	-0.2712	0.3310	-0.1056
23-b	0.3878	0.9186	-0.7363	-0.5308	0.4183	-0.1515
24-a	0.3350	0.4528	-0.5401	0.0617	0.0699	0.7688
24-b	0.4435	0.1012	-0.3784	-0.1082	-0.2199	0.4579
27A-a	0.7964	-0.0195	-0.4094	-0.8598	0.6094	0.2576
27A-b	1.0953	-0.3920	-0.5889	-0.6242	0.4586	-0.3281
28A-a	1.0350	0.5203	-0.5578	-1.5530	0.2125	-0.0576
28A-b	1.5030	0.3510	-0.7023	-0.8128	0.3050	-0.3115
28B-a	1.3202	0.4968	-0.5726	-0.7285	-0.1509	-0.4878
28B-b	1.2640	-0.0706	-0.5102	-0.3396	-0.2521	-0.6710
31-a	0.7933	0.1062	0.1177	-0.5636	-0.3128	-0.6768
31-b	1.2599	0.2095	0.0237	-0.7685	0.2283	-0.2336
32-a	-2.3855	0.0958	-2.0188	0.1969	-0.0774	0.2597
32-b	-2.9169	0.3946	-2.1943	0.4997	0.1445	0.4747
34-a	-1.9025	0.7269	-0.7903	0.3823	-1.0264	-0.1268
34-b	-2.2300	0.8766	-0.4898	0.3492	-1.6571	0.0843
35-a	0.6867	-1.3665	-0.1910	0.5500	-0.7301	-0.0617
35-b	0.7232	-1.9163	-0.7930	0.7333	-0.4579	-0.5068
38A-a	0.8889	-2.6680	-0.7724	1.6084	-0.5115	0.0661
38A-b	0.7855	-2.2376	-0.6248	1.2535	-0.1382	0.5464
39-a	0.3337	0.2073	0.2880	-0.8146	-0.1537	0.4008

TABLE 7: The values of the six canonical variate means for each OTU.

OTU	1	2	3	4	5	6
39-b	0.3145	0.2149	0.3103	-0.7983	-0.3420	0.5586
41-a	0.3283	-0.0169	0.7575	-0.5581	-1.0468	0.5621
41-b	0.3928	-0.1061	0.7379	-0.8735	-0.9663	0.8863
43-a	0.3975	0.2658	0.2372	-1.0338	-0.0514	1.0140
43-b	0.1348	-0.1563	0.3907	-1.0748	-0.4379	0.8107
45-a	0.8645	1.1198	-0.1442	0.4355	0.4123	-0.2289
45-b	1.2673	1.4357	-0.0277	-0.0799	1.2228	-0.2273
47-a	1.0241	0.9157	0.1674	-0.2325	-0.9197	-0.1759
47-b	1.0457	0.8593	0.6629	-0.4626	0.0054	0.2426
48-a	0.8395	0.7655	0.7733	0.6175	-0.8726	-0.2122
48-b	0.8322	0.5613	0.8723	0.8641	-1.1320	-0.1934
50-a	0.6682	0.6851	1.1432	1.2690	-0.8794	-0.2256
50-b	0.5228	0.6175	1.1506	1.1109	-0.5076	0.1959
51-a	-0.3275	-1.1948	-0.0089	-0.7397	0.3943	0.5232
51-b	-0.2168	-1.2832	-0.1099	-0.9485	0.3699	0.6879
52-a	0.3686	-0.2149	0.1287	-0.4346	-0.7209	-0.0673
52-b	0.1637	-0.8795	-0.2174	0.6781	-0.0328	0.1820
53-a	0.2264	1.4756	1.3446	0.4702	-0.4882	-0.7570
53-b	0.2287	1.4817	1/3734	0.9001	0.1991	-1.0075
54-a	-0.0623	0.0619	-0.2438	0.4023	-1.1636	-1.0435
54-b	-0.2149	0.1689	0.2064	-0.4922	-1.0779	-0.9749
56-a	0.4081	1.2583	0.3054	1.1516	1.6511	-0.9593
56-b	0.1458	0.8640	0.4603	-0.0629	1.1036	-0.8170
57-a	-0.2241	-0.0073	1.0609	-0.1951	-0.2442	0.7553
57-b	-0.4665	-0.2910	1.4043	0.2451	-0.8421	0.1784
58-a	0.1277	-0.9479	-1.2746	-0.2758	-0.8656	-0.5063
58-b	0.0605	-0.5307	-1.5970	-0.4406	-0.8438	-0.9871
60-a	0.2955	-0.1962	-1.7192	-0.2895	0.2336	-0.9395
60-b	0.2313	-0.2631	-2.1920	-0.7978	0.2709	-0.9983
71-a	-1.2233	0.3009	0.2962	1.1193	-0.1772	-1.2921
71-b	-1.3922	0.2501	0.6239	0.9018	-0.3722	-1.7611
73-a	-1.4377	0.3882	0.6978	-0.2487	0.0708	0.0309
73-b	-1.3324	0.3825	0.4782	-0.7720	0.0242	-0.1803
74-a	-0.3960	-0.3221	0.1060	-0.2225	-0.2138	0.7544
74-b	-0.1718	-0.1899	-0.0301	0.3436	0.2998	0.8638
75A-a	-1.3454	0.4353	0.6234	0.3794	-0.6076	0.8906
75A-b	-1.2396	0.1980	0.7206	0.2951	-1.0378	0.2570
76A-a	-1.0860	0.7089	-0.2274	-0.3412	-0.0751	0.6056
76A-b	-1.1822	0.1828	-0.8491	-0.8027	-0.1241	0.2698
77-a	-0.2854	-0.6586	0.5843	1.2989	1.6433	-0.2337
77-b	-0.3207	-0.3652	0.3907	0.8131	1.9523	-0.2756
78-a	-1.1182	-0.1587	0.7834	0.3517	0.6889	0.0594
78-b	-1.1328	-0.3644	0.4640	-0.2912	0.7802	-0.5101
78A-a	-0.8550	0.2591	0.7316	-0.4718	0.0560	0.0439
78A-b	-1.0155	0.3475	0.7660	-0.5824	0.0519	-0.4937
81-a	-0.3628	-1.1693	1.4237	-0.5856	0.9074	-0.2206
81-b	-0.3276	-1.3653	1.5613	-0.7334	1.2005	0.1692
82-a	-1.0792	-1.8433	1.4538	-1.4396	-0.1117	-0.9015
82-b	-1.0898	-2.4209	1.6901	-1.0488	0.1550	-0.6279
83-a	1.2203	-0.1977	-0.2243	1.3437	-0.4429	0.2974
83-b	1.1084	-0.4254	0.0238	1.2969	-0.4832	0.6890
84-a	0.4520	-0.8302	-0.4055	0.2452	0.7753	0.5940
84-b	0.1508	-0.8532	-0.4275	0.8950	1.3217	0.5575

TABLE 7 (cont.): The values of the six canonical variate means for each OTU.

OTU	1-a	1-b	3-a	3-b	4-a	4-b	9-a	9-b	14A-a	14A-b	16-a	16-b	18-a	18-b	20-a	20-b	20A-a	20A-b	23-a	23-b	24-a	24-b	27A-a	27A-b	28A-a	28A-b	28B-a	28B-b	31-a	31-b	32-a	32-b	34-a	34-b
1-a		NS																																
1-b	0.8562			NS	NS	NS				NS	NS	NS				NS					NS													
3-a	2.3444	2.1999		NS			NS				NS	NS	NS	NS					NS					NS			NS	NS	NS	NS				
3-b	1.9023	1.5281	1.0684		NS	NS					NS	NS	NS	NS					NS			NS	NS	NS						NS				
4-a	2.0882	1.4554	1.6579	1.3137		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS			NS		NS	NS				
4-b	2.1230	1.3961	1.8398	1.2886	0.5109		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS			NS	NS	NS	NS	NS	NS										
9-a	2.2826	1.9527	1.4224	1.5960	1.0221	1.3052		NS	NS	NS	NS	NS	NS	NS	NS	NS			NS	NS	NS	NS	NS	NS		NS	NS	NS	NS	NS				
9-b	2.3931	1.9684	1.6438	1.6257	1.0448	1.2179	0.4731		NS	NS	NS	NS	NS	NS	NS	NS			NS	NS	NS	NS	NS		NS		NS		NS	NS				
14A-a	2.1180	1.5619	2.2249	1.9019	0.9862	1.0383	1.0762	0.8610		NS	NS	NS	NS	NS	NS				NS	NS	NS	NS	NS											
14A-b	2.1330	1.7170	1.9387	1.8083	1.1218	1.1507	0.8058	0.7125	0.6409		NS	NS	NS	NS	NS	NS			NS	NS	NS	NS												
16-a	1.8526	1.3695	1.5822	1.3865	0.7382	0.8391	0.9135	0.8591	0.9021	0.7251		NS	NS	NS	NS	NS			NS	NS	NS	NS	NS											
16-b	1.8990	1.5462	1.3919	1.3218	1.0347	1.0526	0.9555	0.9761	1.1600	0.7119	0.5117		NS	NS	NS	NS			NS	NS	NS	NS	NS											
18-a	2.0093	1.5928	1.3325	1.3018	0.6405	0.9196	0.5028	0.7457	1.0225	0.8166	0.6046	0.6202		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS			NS	NS	NS	NS				
18-b	2.1572	1.7780	1.2088	1.2736	0.8982	1.0329	0.7346	0.9938	1.3462	0.9924	0.8209	0.5675	0.4330		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS		NS	NS	NS	NS	NS				
20-a	2.7447	2.3167	1.7186	1.9186	1.0634	1.5286	1.0127	1.1879	1.5348	1.5815	1.3718	1.5860	1.0493	1.2975		NS	NS	NS	NS		NS	NS					NS	NS	NS					
20-b	2.6010	2.1744	1.3842	1.6143	0.8934	1.2565	0.6809	0.9327	1.4182	1.2638	1.0884	1.1414	0.6615	0.7600	0.6035		NS	NS	NS	NS	NS	NS	NS	NS		NS	NS	NS	NS	NS				
20A-a	2.2841	2.0829	1.6787	1.8633	1.4596	1.0969	1.6511	1.9936	2.1576	2.1677	1.7535	1.9915	1.4327	1.5707	1.2498	1.2585		NS			NS	NS												
20A-b	2.9427	2.6593	2.1423	2.3670	1.8512	2.3264	2.0657	2.3139	2.5066	2.6300	2.2147	2.4636	1.9587	2.1666	1.3651	1.6747	0.9033					NS												
23-a	2.1486	1.8555	1.5490	1.5393	1.2561	1.3455	0.6084	0.6649	1.1176	0.7515	1.0862	0.8309	0.7629	0.8357	1.5727	1.1708	2.0212	2.5607		NS	NS	NS	NS		NS	NS	NS		NS	NS				
23-b	2.4435	2.1002	1.6724	1.6603	1.3792	1.4004	0.8100	0.8129	1.2897	0.9200	1.2984	1.0077	0.9484	0.9045	1.6909	1.2309	2.2032	2.7346	0.3737		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS				
24-a	2.0104	1.4239	1.9882	1.6345	0.5999	0.8116	1.0254	1.0246	0.7240	1.0053	0.9758	1.1952	0.7853	1.1056	1.2667	1.1508	1.6278	2.0736	1.1182	1.2560		NS	NS				NS							
24-b	2.3837	1.8221	1.7462	1.5776	0.5023	0.9165	0.9050	0.9585	1.0729	1.2369	1.0733	1.2948	0.7304	1.0234	0.7487	0.7049	1.3822	1.6748	1.2366	1.3254	0.6092		NS	NS			NS	NS	NS	NS				
27A-a	2.8391	2.2259	1.8075	1.4511	1.1910	1.0394	1.4592	1.3563	1.6522	1.6027	1.5719	1.5052	1.2953	1.2119	1.7085	1.3170	2.2238	2.6109	1.3918	1.2107	1.3618	1.1971		NS	NS	NS	NS	NS	NS	NS				
27A-b	3.1343	2.6404	1.6475	1.6628	1.5231	1.5557	1.6334	1.7397	2.1902	2.0237	1.9021	1.7646	1.4871	1.2734	1.6883	1.2668	2.0045	2.4050	1.6955	1.5105	1.7661	1.4342	0.8256		NS	NS	NS	NS	NS	NS				
28A-a	3.5222	3.0087	2.2772	2.2043	1.9590	1.9133	1.7150	1.5315	2.0462	1.9387	2.2077	2.0646	1.8574	1.8064	2.0651	1.7344	2.8499	3.1205	1.5947	1.3059	1.9507	1.7601	1.0524	1.3540		NS	NS	NS	NS	NS				
28A-b	3.2623	2.8462	1.9689	1.9936	1.8613	1.9155	1.6565	1.7132	2.2187	2.0711	2.2075	2.0121	1.6976	1.5756	1.9317	1.5674	2.3275	2.7418	1.5516	1.2982	1.8407	1.6292	1.0683	0.8892	0.9431		NS	NS	NS	NS				
28B-a	3.2475	2.8862	1.8269	2.0460	1.8216	1.9992	1.3974	1.4928	2.1218	1.9684	2.1224	1.9584	1.5733	1.5273	1.6185	1.3328	2.1418	2.4645	1.4277	1.2451	1.7961	1.4989	1.3109	1.1175	1.0388	0.5635		NS	NS	NS				
28B-b	3.2278	2.8774	1.5895	1.9592	1.7294	1.9926	1.4790	1.7071	2.3048	2.1462	2.0845	1.9702	1.5307	1.4430	1.3702	1.1246	1.6906	1.9817	1.6986	1.5979	1.8643	1.4315	1.4514	0.9174	1.5710	0.9671	0.7238		NS	NS				
31-a	3.0753	2.7222	1.2283	1.6491	1.6056	1.8759	1.2605	1.3649	2.1027	1.9652	1.8487	1.7860	1.4055	1.4148	1.1962	1.0205	1.7996	1.9341	1.5510	1.5380	1.8413	1.3683	1.4509	1.2486	1.5246	1.3466	0.9979	0.8372		NS				
31-b	3.0291	2.5796	1.5556	1.5051	1.5873	1.6788	1.5123	1.4970	2.0716	2.0105	1.9758	1.8728	1.5257	1.4926	1.6541	1.3790	2.0990	2.3904	1.5200	1.3930	1.7180	1.3982	0.9218	0.9203	1.0640	0.7875	0.8073	0.9846	0.8765					
32-a	3.6220	3.4052	3.0085	3.8953	3.1079	3.1325	3.0239	3.0922	2.8106	2.5728	2.6079	2.7058	2.9661	2.9893	3.2669	3.1188	3.6696	3.9655	3.2018	3.3102	3.1645	3.2934	3.7838	3.9630	4.1547	4.2908	4.1719	4.0996	4.0225	4.3292		NS		
32-b	3.8436	3.7213	4.3556	4.3940	3.6774	3.6717	3.6067	3.6622	3.2984	3.0875	3.1382	3.2281	3.5369	3.5729	3.9163	3.7725	4.2242	4.5564	3.7211	3.8536	3.6876	3.8959	4.3883	4.6152	4.7763	4.9123	4.8078	4.7651	4.6755	4.9514	0.7679			
34-a	3.1238	3.0375	3.0753	3.3588	2.6542	2.9004	2.2801	2.3683	2.3115	2.1575	2.1634	2.3432	2.4137	2.6068	2.4210	2.4870	2.9234	3.0139	2.6293	2.8647	2.6928	2.7014	3.5146	4.0740	3.7430	3.8706	3.5520	3.4868	3.1912	3.7203	1.7958	2.3497		NS
34-b	3.6430	3.5578	3.6486	3.9148	3.1892	3.4601	2.8504	2.8758	2.7950	2.7675	2.7799	3.0205	3.0216	3.2713	2.8490	3.0414	3.4142	3.3172	3.2269	3.4717	3.2086	3.1899	3.6794	4.2744	4.2336	4.4288	4.0635	4.0155	3.6501	4.2182	2.2039	2.6514	0.8145	
35-a	3.3612	2.9373	2.2695	2.4454	1.8982	2.2556	2.3175	2.5492	2.7487	2.8055	2.3449	2.5529	2.1066	2.1617	1.4947	1.7061	1.3060	1.0383	2.7807	2.8466	2.2635	1.7921	2.3996	2.0341	3.0210	2.6208	2.4839	1.8727	2.0169	2.3547	3.9460	4.5995	3.4008	3.8143
35-b	3.8316	3.4655	2.6687	2.9489	2.4984	2.7675	2.8470	3.1400	3.3509	3.2774	2.8364	2.9379	2.6071	2.5181	2.2067	2.2254	1.8712	1.8762	3.2512	3.2626	2.8609	2.4528	2.8306	2.2844	3.4595	2.9611	2.9085	2.2360	2.5801	2.8766	4.0293	4.6835	3.8042	4.3068
38A-a	4.3386	4.0026	3.6946	3.7912	3.3382	3.5778	3.8808	4.1595	4.1827	4.2215	3.7181	3.8981	3.5707	3.5399	3.1916	3.3071	2.5884	2.5088	4.2633	4.3133	3.6503	3.3478	3.8132	3.3671	4.5570	4.0210	4.0174	3.3707	3.7158	3.9164	4.7045	5.2642	4.5960	5.0270
38A-b	3.7414	3.3072	3.2674	3.1810	2.6783	2.8655	3.3670	3.5935	3.5314	3.6263	3.1103	3.3259	3.0038	2.9972	2.7293	2.8180	2.1838	2.2031	3.7066	3.7566	2.9936	2.7416	3.1741	2.8548	4.0046	3.5247	3.5726	2.9951	3.2975	3.3859	4.3180	4.8736	4.2476	4.7062
39-a	2.7602	2.1708	1.7765	1.5226	1.0858	1.2119	1.2489	0.9770	1.3549	1.5262	1.4183	1.5970	1.2672	1.4870	1.2340	1.1941	2.0906	2.1671	1.4646	1.5069	1.3035	0.9869	1.1648	1.6281	1.4814	1.7569	1.6108	1.7216	1.2247	1.2158	3.7117	4.3110	2.9842	3.3675
39-b	2.8251	2.2244	1.9635	1.7085	1.1451	1.3083	1.3434	1.0651	1.3469	1.6021	1.5076	1.7410	1.3773	1.6394	1.2149	1.2839	2.1186	2.1219	1.5978	1.6582	1.3166	1.0026	1.3434	1.8011	1.6195	1.9059	1.7340	1.8403	1.3640	1.3891	3.7254	4.3215	2.9531	3.2899
41-a	3.2070	2.6906	2.3165	2.2312	1.7002	2.0061	1.8782	1.7289	1.9757	2.2776	2.0736	2.3815	1.9451	2.2481	1.3142	1.6994	2.1453	1.7816	2.2820	2.4014	1.8917	1.4881	2.1231	2.3674	2.3404	2.5104	2.2250	2.1646	1.6476	1.9392	4.0849	4.6861	3.0471	3.2130
41-b	3.4108	2.8086	2.6033	2.3868	1.8076	2.0207	2.1010	1.8769	2.0306	2.3922	2.2327	2.5506	2.1394	2.4275	1.5950	1.9184	2.4632	2.1336	2.4424	2.5169	1.9777	1.6180	2.0892	2.4296	2.2860	2.5633	2.3484	2.3538	1.8859	2.0133	4.2057	4.8054	3.3023	3.4595
43-a	2.9172	2.2217	2.3566	1.8770	1.3168	1.2787	1.6937	1.3451	1.3666	1.7224	1.7101	1.9392	1.6565	1.8896	1.6977	1.6919	2.5055	2.5767	1.7848	1.7906	1.3850	1.2660	1.3030	1.9574	1.6096	2.0113	1.9797	2.1873	1.8289	1.5804	3.8663	4.4266	3.2888	3.6278
43-b	3.2002	2.5258	2.3588	2.0600	1.4371	1.5188	1.8018	1.5112	1.6489	1.9464	1.8023	2.0941	1.7878	2.0174	1.5115	1.6310	2.4585	2.3359	2.0787	2.1081	1.6818	1.3636	1.5954	2.0594	1.8903	2.2812	2.1559	2.2056	1.7512	1.7783	3.7778	4.3851	3.1103	3.3877
45-a	1.8417	1.7944	1.4423	1.4284	1.6168	1.8074	1.2625	1.4714	1.7988	1.6603	1.6693	1.4862	1.2507	1.3335	1.8560	1.6077	1.7052	2.3568	1.0601	1.2482	1.4610	1.5610	1.8238	1.9161	2.1408	1.6991	1.5869	1.7170	1.6809	1.5801	3.9574	4.4371	3.2111	3.7607
45-b	2.3209	2.2382	1.9510	1.6752	2.2522	2.2111	2.0099	2.0399	2.3512	2.1906	2.3059	2.0202	1.9825	1.9318	2.7060	2.3504	2.6624	3.3009	1.5549	1.5489	2.1069	2.2659	1.9247	2.1390	2.0958	1.7533	1.8859	2.2229	2.1925	1.7232	4.5952	5.0275	4.0514	4.6186
47-a	2.9882	2.7325	1.9674	2.1858	1.8730	2.2354	1.4079	1.4648	2.0024	2.0735	2.1474	2.1871	1.6913	1.9310	1.3533	1.5111	1.8993	1.9375	1.6591	1.7611	1.7743	1.4850	2.0441	2.0889	1.9271	1.7775	1.3210	1.4804	1.1999	1.4776	4.2615	4.8448	3.1480	3.4602
47-b	2.6045	2.2281	1.7892	1.5132	1.6584	1.8084	1.5848	1.4474	1.8565	2.0026	1.9782	2.0091	1.6561	1.8576	1.7801	1.7419	2.1714	2.3697	1.5695	1.6506	1.6351	1.4981	1.5834	1.9214	1.7109	1.6885	1.5368	1.7901	1.3728	1.1165	4.4709	5.0080	3.5687	3.9379
48-a	2.5986	2.5171	1.8752	2.0956	2.0487	2.4646	1.8196	1.9717	2.3489	2.4554	2.2667	2.3803	1.9293	2.1988	1.6180	1.8770	1.5619	1.5293	2.1143	2.3442	2.0585	1.8238	2.5723	2.5668	2.7897	2.4964	2.1268	2.0095	1.6706	2.0448	4.4359	4.9574	3.1704	3.4359
48-b	2.7835	2.7170	2.1210	2.3649	2.2540	2.6983	2.1099	2.2842	2.6075	2.7411	2.4947	2.6526	2.1866	2.4597	1.7406	2.0833	1.5605	1.3493	2.4613	2.6951	2.2953	2.0319	2.8170	2.8065	3.1235	2.8044	2.4322	2.2245	1.9290	2.3546	4.5488	5.0691	3.2432	3.4569
50-a	2.5499	2.6246	2.1877	2.3853	2.4660	2.8766	2.3660	2.5503	2.8086	2.9247	2.6174	2.7658	2.3860	2.6468	2.1274	2.4048	1.7135	1.6355	2.6570	2.9316	2.5192	2.3464	3.1189	3.1473	3.4984	3.1557	2.8273	2.6152	2.2990	2.6847	4.6582	5.1193	3.3417	3.5567
50-b	2.1377	2.1218	2.0528	2.0068	2.1009	2.4621	2.1784	2.2992	2.4436	2.6275	2.2738	2.4721	2.1189	2.4090	1.9977	2.2420	1.6269	1.6498	2.4241	2.7097	2.1642	2.0612	2.9312	2.8372	3.2969	3.0140	2.7564	2.5888	2.2372	2.4975	4.4498	4.8935	3.2505	3.5001
51-a	3.1997	2.4992	2.2913	1.9759	1.5312	1.4162	2.2006	2.1060	2.1463	2.1952	1.7431	1.9836	1.9191	1.9116	1.9489	1.8096	2.4776	2.5481	2.4548	2.4509	2.0600	1.7864	1.7136	1.7215	2.4762	2.6328	2.6859	2.4601	2.2218	2.2570	3.3331	3.9507	3.2379	3.7052
51-b	3.4145	2.6774	2.5245	2.1792	1.6672	1.5202	2.3489	2.2254	2.2377	2.3211	1.9393	2.1744	2.0844	2.0780	2.0774	1.9491	2.6629	2.7094	2.5846	2.5469	2.1495	1.8793	1.9358	1.9724	2.4426	2.6459	2.7289	2.5353	2.3408	2.3083	3.4560	4.0796	3.4248	3.8755
52-a	3.0167	2.5396	1.6670	1.8386	1.2590	1.6287	1.2688	1.2981	1.7828	1.8424	1.6075	1.7965	1.3275	1.5152	0.6067	0.8527	1.6010	1.4606	2.3491	2.6185	1.5700	0.9980	1.6021	1.6030	1.8915	1.8849	1.5786	1.3506	0.9156	1.4232	3.6350	4.2986	2.7666	3.1370
52-b	2.4485	1.9991	1.7879	1.7464	1.1877	1.4955	1.8008	2.0257	2.0690	2.0977	1.5089	1.7538	1.4319	1.4976	1.3244	1.3412	0.9948	1.2860	2.4886	2.7607	1.6259	1.3399	1.9950	1.8550	2.8159	2.4746	2.4080	1.9396	1.9591	2.1861	3.3057	3.8949	2.8893	3.4123
53-a	2.6519	2.7951	1.9838	2.2972	2.6823	2.9792	2.2188	2.2859	2.7497	2.7101	2.5825	2.5826	2.4081	2.6148	2.4389	2.5149	2.4943	2.5915	1.7605	1.8296	2.7233	2.6052	3.1048	3.2030	3.2029	3.0916	2.7300	2.7616	2.1927	2.5956	4.6169	5.0488	3.2164	3.4419
53-b	2.3539	2.6796	1.8886	2.1779	2.8637	3.0969	2.4732	2.6031	3.0191	2.8934	2.6925	2.6058	2.5517	2.6705	2.8261	2.7761	2.5876	2.8889	2.1803	2.3084	2.9337	2.8915	3.2589	3.3114	3.4963	3.2621	2.9989	2.9805	2.5103	2.8085	4.7369	5.1162	3.5127	3.8502
54-a	3.1483	3.0282	1.8108	2.5200	2.1135	2.5561	1.6138	1.9416	2.4550	2.2643	2.0670	2.1146	1.7937	1.8954	1.2690	1.4223	1.5829	1.5281	2.1499	2.3125	2.3066	1.9185	2.6834	2.4212	2.8638	2.6293	2.1956	1.8351	1.6298	2.4094	3.3866	4.0111	2.2331	2.6347
54-b	3.4004	3.1521	1.7762	2.4009	2.1144	2.4634	1.5688	1.6689	2.3429	2.1720	2.0512	2.1012	1.8521	1.9630	1.3491	1.4454	2.1751	2.0210	2.0634	2.1753	2.3675	1.9278	2.4370	2.3367	2.4293	2.5031	2.0551	1.8656	1.3068	2.1312	3.5592	4.2064	2.3747	2.6854
56-a	1.8190	2.2595	1.8968	1.9177	2.7964	2.8290	2.5775	2.7812	3.0324	2.7396	2.5544	2.2581	2.4401	2.3375	3.2122	2.8767	2.7025	3.4386	2.3141	2.4907	2.8361	2.9840	2.9843	2.9949	3.4468	3.0213	3.0310	3.0154	2.8964	2.8535	4.4657	4.7751	3.9804	4.5183
56-b	2.1181	2.0824	0.9927	1.0737	2.0129	2.0159	1.7003	1.7619	2.2519	1.9292	1.7628	1.4581	1.6569	1.5516	2.3697	1.9840	2.4100	2.9520	1.4895	1.6201	2.1919	2.1801	1.9986	2.1331	2.3529	2.2164	2.1693	2.2364	1.8404	1.8613	3.9705	4.4217	3.3151	3.8924
57-a	2.5234	1.9887	2.0998	1.7208	1.4965	1.6620	1.9337	1.7607	1.7909	2.0930	1.6809	2.0475	1.8190	2.1127	1.6970	1.8837	2.1701	2.0572	2.2188	2.4157	1.8034	1.6204	2.1498	2.5421	2.6827	2.8358	2.6732	2.5966	2.0320	2.2053	3.8203	4.3269	2.9167	3.1549
57-b	2.9522	2.6411	2.2424	2.2684	2.1374	2.4453	2.3506	2.3320	2.5221	2.7004	2.2220	2.5551	2.3048	2.5678	1.8814	2.2026	2.1760	1.7821	2.7798	3.0124	2.4881	2.1795	2.8797	3.0210	3.3412	3.3770	3.0969	2.8496	2.2508	2.7349	4.0179	4.5333	2.8392	2.9572
58-a	3.7435	3.3147	2.4453	2.8379	2.0148	2.2963	1.9626	2.1908	2.5024	2.3362	2.1861	2.2556	1.9473	1.8914	1.4614	1.4118	2.0371	2.0575	2.4252	2.3834	2.2714	1.8380	2.2711	1.9031	2.5470	2.3670	2.1721	1.7472	1.9774	2.4126	3.0644	3.8081	2.7864	3.2969
58-b	3.9084	3.5728	2.5120	3.0491	2.3435	2.5985	1.9777	2.2342	2.6593	2.3524	2.3633	2.3009	2.0847	1.9660	1.7831	1.6021	2.3784	2.5183	2.3680	2.2908	2.5160	2.1491	2.4597	2.0665	2.5201	2.3608	2.1223	1.8169	2.0679	2.5372	3.0169	3.7518	2.7452	3.2994
60-a	3.3606	3.0661	2.1244	2.5325	2.0837	2.1871	1.7459	2.0331	2.4198	1.9754	2.0300	1.7681	1.7229	1.4147	2.0176	1.5373	2.3108	2.8197	1.8612	1.7119	2.2094	2.0247	1.9741	1.5786	2.1871	1.8614	1.8410	1.6510	2.0387	2.2045	3.0223	3.6777	3.0407	3.7512
60-b	3.9368	3.5956	2.7200	3.0762	2.5802	2.6079	2.2460	2.4398	2.7930	2.3494	2.5171	2.2579	2.2693	1.9748	2.5269	2.0679	2.9735	3.4173	2.3046	2.0857	2.6747	2.5108	2.2917	1.9616	2.3214	2.1649	2.1975	2.1266	2.5072	2.6035	3.1144	3.7700	3.3664	4.0570
71-a	2.6069	2.8031	1.9667	2.5587	2.6634	2.9439	2.3253	2.5891	2.8956	2.6017	2.2187	2.2139	2.3215	2.3596	2.4686	2.4079	2.3162	2.5547	2.6296	2.9020	2.9288	2.8008	3.4084	3.3186	3.8333	3.6551	3.6352	3.0814	2.7140	3.3313	3.1655	3.5620	2.1085	2.5769
71-b	3.1852	3.3621	2.2272	2.9207	3.1185	3.3952	2.7192	2.9393	3.3523	3.0461	2.6955	2.6719	2.7739	2.7934	2.8074	2.7658	2.7774	2.8846	3.0312	3.2762	3.4178	3.2193	3.7514	3.6254	4.0781	3.9562	3.6301	3.3449	2.8949	3.5888	3.5583	3.9642	2.4201	2.7743
73-a	2.5129	2.2374	2.1233	2.0836	2.0083	2.0836	1.9682	1.8344	1.9420	1.8783	1.5506	1.7691	1.9549	2.1301	1.1937	1.1464	2.7160	2.8151	2.1924	2.4297	2.3064	2.2507	2.6642	3.0167	3.0720	3.3316	3.1273	3.0942	2.4780	2.8510	2.9388	3.3637	2.0425	2.3712
73-b	2.8776	2.5375	2.0950	2.1482	2.0270	2.0690	1.8441	1.6516	1.9224	1.7713	1.5900	1.7206	1.9174	2.0374	2.1304	2.0114	2.8648	2.9573	2.0571	2.2214	2.3280	2.2079	2.4544	2.8047	2.7124	3.0874	2.8776	2.9032	2.2648	2.6460	2.9274	3.4225	2.1192	2.4778
74-a	2.5135	1.8612	2.0678	1.7756	0.9015	1.0815	1.5356	1.4218	1.2991	1.5541	1.1065	1.5578	1.3259	1.5887	1.2383	1.3420	1.8925	1.9125	1.8990	2.0441	1.3091	1.1043	1.7634	2.1206	2.4102	2.5430	2.4247	2.2903	1.9423	2.1215	3.0144	3.5900	2.4446	2.8310
74-b	1.8495	1.1490	1.9516	1.4480	0.6869	0.7984	1.5680	1.5712	1.2647	1.5009	0.9295	1.3506	1.1649	1.4015	1.5036	1.4701	1.6078	1.9409	1.7781	1.9615	1.0345	1.1059	1.7382	2.0836	2.5867	2.5034	2.4839	2.3308	2.1476	2.1580	3.0766	3.5721	2.6747	3.1655
75A-a	2.4196	2.1217	2.7435	2.5711	2.0448	2.2475	2.2271	2.1291	1.8691	2.1240	1.8085	2.2323	2.1759	2.5149	2.1501	2.3701	2.5524	2.4902	2.5458	2.8377	2.1800	2.2114	3.0460	3.4263	3.5177	3.6847	3.4460	3.3810	2.8933	3.2369	2.9817	3.3412	1.8986	1.9923
75A-b	2.1866	2.5770	2.5209	2.6251	2.1559	2.4637	2.2158	2.1294	2.1661	2.2790	1.9564	2.3212	2.1892	2.4939	1.8930	2.1851	2.3807	2.1483	2.5984	2.8739	2.3826	2.2185	3.0849	3.3077	3.4626	3.5950	3.2715	3.1203	2.5768	3.1157	3.1241	3.5827	1.7769	1.8229
76A-a	2.3235	1.8920	2.3439	2.1695	1.4986	1.5496	1.4404	1.2258	0.9517	1.0073	1.0379	1.3397	1.4574	1.7228	1.8282	1.7570	2.5279	2.7465	1.6080	1.8241	1.5467	1.6818	2.2284	2.7087	2.5757	2.8749	2.7028	2.8055	2.4010	2.6066	2.3839	2.8446	1.7172	2.1580
76A-b	3.0190	2.5342	2.5114	2.5128	1.7142	1.7171	1.6014	1.4574	1.4193	1.2215	1.3227	1.4828	1.6342	1.7230	1.8830	1.6919	2.7515	2.9503	1.8297	1.8880	1.8719	1.8434	2.1657	2.5127	2.4286	2.7897	2.6490	2.6979	2.4150	2.6655	1.9558	2.5834	1.7872	2.3280
77-a	2.0429	2.0061	2.0563	1.7607	2.4337	2.4089	2.8767	3.0455	3.0753	2.9440	2.3161	2.3186	2.4754	2.3812	3.0093	2.7804	2.3953	2.9288	2.9182	3.0886	2.8099	2.8223	2.9218	2.9094	3.8274	3.4828	3.5560	3.2269	3.0754	3.1196	4.0218	4.3751	3.7952	4.3804
77-b	1.9977	1.9002	1.9174	1.5251	2.3235	2.1915	2.6938	2.8131	2.8686	2.6677	2.1251	2.0290	2.3078	2.1538	3.0133	2.6775	2.5960	3.1976	2.6251	2.7558	2.6766	2.7383	2.6256	2.6953	3.4920	3.2237	3.3479	3.1231	2.9537	2.9049	3.8816	4.2342	3.7642	4.3971
78-a	2.0617	1.7926	1.8480	1.6175	1.8657	1.8876	2.1663	2.1607	2.1883	2.1234	1.1530	1.7157	1.9207	2.0071	2.3081	2.1925	3.3789	2.6221	2.3431	2.5757	2.2820	2.2478	2.5732	2.8271	3.2809	3.3135	3.2227	3.0418	2.5594	2.8144	3.1897	3.5914	2.6180	3.0714
78-b	2.6772	2.3621	1.6721	1.7089	1.9905	1.9574	2.0913	2.0790	2.3420	2.0910	1.5956	1.6223	1.9216	1.8545	2.3107	2.0453	2.6220	2.8725	2.2606	2.3907	2.4688	2.3199	2.3549	2.5142	2.9403	3.0589	2.9819	2.8060	2.3126	2.6186	3.0841	3.5812	2.6872	3.2183
78A-a	2.5092	2.1161	1.7516	1.6585	1.6235	1.7097	1.6356	1.4566	1.7099	1.6893	1.3438	1.5597	1.6230	1.8031	1.8033	1.7448	2.4217	2.5017	1.8766	2.0753	1.9700	1.8229	2.1469	2.5090	2.5508	2.8159	2.6230	2.6008	1.9445	2.2740	3.2319	3.7373	2.3585	2.7156
78A-b	2.7839	2.5151	1.7024	1.8763	2.0122	2.1119	1.7828	1.6630	2.0895	1.9153	1.6437	1.7185	1.8569	1.9547	2.0344	1.9149	2.6454	2.7350	2.1021	2.1922	2.3558	2.1687	2.3982	2.6519	2.6729	2.9409	2.7077	2.6799	1.9792	2.4250	3.2995	3.8076	2.3624	2.7303
81-a	3.3689	2.9136	2.1518	1.9055	2.4649	2.4023	2.8914	2.8189	3.0971	3.0635	2.5150	2.6255	2.6670	2.6282	2.7712	2.6379	2.9780	3.0106	3.0698	3.1361	3.0123	2.7334	2.5336	2.6448	3.1907	3.2765	3.2871	3.0334	2.5232	2.6437	4.3995	4.9298	3.9438	4.3448
81-b	3.5698	3.0519	2.5973	2.1711	2.7035	2.5634	3.2498	3.1350	3.3135	3.3389	2.8038	2.9381	2.9894	2.9574	3.1321	3.0017	3.3292	3.3676	3.3819	3.4297	3.2296	3.0000	2.7077	2.8991	3.4062	3.5299	3.6047	3.3870	2.9224	2.9109	4.6577	5.1609	4.3220	4.7115
82-a	4.7633	4.3176	3.1683	3.3443	3.4929	3.5330	3.6684	3.5892	3.9928	3.9051	3.4768	3.6027	3.6113	3.5755	3.3517	3.3260	3.9020	3.6463	4.0024	4.0306	4.0404	3.6179	3.5378	3.5109	3.8644	4.1267	4.0022	3.7113	3.1545	3.5697	4.6425	5.2358	4.1323	4.3658
82-b	4.8664	4.4113	3.4725	3.5290	3.7178	3.7371	4.0705	4.0178	4.3181	4.2788	3.7530	3.9194	3.9222	3.8862	3.6843	3.6802	4.0419	3.7799	4.4026	4.4483	4.2958	3.8981	3.8418	3.8009	4.3361	4.5065	4.4336	4.0714	3.5863	3.9315	4.9156	5.4743	4.5185	4.7615
83-a	2.4985	2.2939	2.2643	2.2564	1.8436	2.2327	2.1548	2.4464	2.4900	2.6214	2.2898	2.4394	1.9362	2.0962	1.7363	1.9084	0.7771	1.1877	2.4291	2.5803	1.8535	1.7029	2.4920	2.2941	3.0996	2.4880	2.3684	1.9767	2.2350	2.3286	4.2140	4.7367	3.5173	3.9545
83-b	2.5471	2.2368	2.4559	2.2976	1.8184	2.1849	2.2389	2.5473	2.4920	2.7242	2.3295	2.5698	2.0699	2.2749	1.7988	2.0475	1.0117	1.1504	2.6266	2.7862	1.8777	1.7258	2.5458	2.4472	3.2251	2.7128	2.6169	2.2397	2.3972	2.4595	4.2674	4.7824	3.5852	3.9682
84-a	2.4099	1.7658	1.9903	1.5091	1.1096	1.0325	1.9659	2.0619	1.9759	2.0226	1.5369	1.6824	1.5005	1.4499	1.8487	1.6235	1.7108	2.1769	2.0886	2.1111	1.4966	1.4150	1.4621	1.5316	2.4822	2.1791	2.3492	2.0603	2.1726	1.9822	3.5146	4.0652	3.4497	4.0374
84-b	2.1119	1.6505	2.2555	1.7605	1.7077	1.5998	2.4499	2.6020	2.4209	2.3951	1.8492	1.9364	1.9503	1.8596	2.4838	2.2372	2.0016	2.6058	2.4942	2.5772	2.0145	2.0957	2.1884	2.2289	3.2073	2.8390	3.0179	2.7145	2.8201	2.6742	3.5212	3.9545	3.6179	4.2496

TABLE 8: The matrix of values of the Mahalanobis Distances between each group pair below the diagonal, with the significance of each distance above the diagonal (where NS = not significant, otherwise $p < 0.05$).

OTU	35-a	35-b	38A-a	38A-b	39-a	39-b	41-a	41-b	43-a	43-b	45-a	45-b	47-a	47-b	48-a	48-b	50-a	50-b	51-a	51-b	52-a	52-b	53-a	53-b	54-a	54-b	56-a	56-b	57-a	57-b	58-a	58-b	60-a	60-b
1-a											NS																						NS	
1-b											NS													NS	NS	NS		NS						
3-a											NS																	NS						
3-b					NS	NS					NS																		NS					
4-a					NS	NS			NS	NS	NS								NS	NS														
4-b					NS	NS			NS	NS	NS								NS	NS														
9-a					NS	NS			NS		NS		NS								NS				NS	NS								
9-h					NS	NS			NS	NS	NS		NS								NS				NS	NS								
14A-a					NS	NS			NS		NS																							
14A-b					NS				NS		NS																							
16-a					NS	NS			NS		NS											NS			NS	NS								
16-b					NS						NS															NS		NS						
18-a					NS	NS			NS		NS										NS	NS			NS	NS								
18-b					NS						NS										NS	NS			NS	NS		NS					NS	
20-a	NS				NS	NS	NS		NS	NS	NS		NS								NS	NS			NS	NS					NS			
20-b					NS	NS			NS	NS	NS		NS								NS	NS			NS	NS					NS		NS	
20A-a	NS										NS				NS	NS		NS			NS	NS			NS									
20A-b	NS	NS					NS				NS				NS	NS	NS	NS			NS	NS			NS	NS			NS					
23-a					NS						NS	NS																NS						
23-b					NS						NS	NS																NS						
24-a					NS	NS			NS		NS										NS													
24-b					NS	NS	NS		NS	NS	NS		NS								NS	NS												
27A-a					NS	NS			NS	NS	NS									NS	NS													
27A-b					NS						NS										NS													NS
28A-a					NS				NS		NS																							
28A-b					NS						NS																							
28B-a					NS				NS		NS		NS	NS							NS				NS	NS								NS
28B-b					NS						NS		NS								NS				NS	NS								
31-a					NS	NS					NS		NS	NS							NS													
31-b					NS	NS					NS		NS	NS							NS													NS
32-a																																		
32-b																																		
34-a																																		
34-b																																		
35-a		NS	NS																															
35-b	0.9859		NS	NS																														
38A-a	1.8046	1.2999		NS																														
38A-b	1.4738	1.2718	0.8449																															
39-a	2.2889	3.0236	3.9763	3.3636		NS	NS	NS	NS	NS	NS		NS	NS							NS					NS			NS					
39-b	2.2900	3.0694	3.9846	3.3709	0.2481		NS	NS	NS	NS	NS		NS	NS							NS					NS			NS					
41-a	2.1369	3.0537	3.8613	3.3405	1.0771	1.0971		NS	NS	NS			NS								NS					NS			NS	NS				
41-b	2.3491	3.2420	4.0162	3.4467	0.8991	0.8915	0.4728		NS	NS											NS					NS			NS					
43-a	2.6566	3.3956	4.2379	3.5523	0.6659	0.6017	1.3329	1.1260		NS	NS			NS						NS	NS								NS					
43-b	2.3651	3.1386	3.9987	3.3715	0.7063	0.5707	0.9439	0.7177	0.6805										NS	NS	NS					NS			NS					
45-a	2.7498	3.2530	4.1302	3.6170	1.8923	2.0098	2.4768	2.7131	2.2387	2.5557		NS	NS	NS	NS	NS	NS	NS	NS		NS	NS	NS	NS	NS	NS	NS	NS						
45-b	3.5290	3.9600	4.8459	4.2749	2.3041	2.4687	3.1002	3.2233	2.5040	2.9693	1.0946																	NS						
47-a	2.4722	3.2076	4.1664	3.7374	1.5003	1.4861	1.5391	1.8192	1.9057	1.4776	1.5451	2.2324		NS	NS	NS					NS				NS	NS								
47-b	2.7333	3.4834	4.3720	3.7914	1.1165	1.1863	1.5842	1.7003	1.3705	1.6820	1.3952	1.6455	1.1545																					
48-a	2.3550	3.1514	3.9205	3.5349	1.9356	1.9167	1.6988	2.0974	2.3744	2.3648	1.6286	2.4802	1.0724	1.4859		NS	NS	NS					NS	NS	NS	NS								
48-b	2.2684	3.0812	3.7607	3.4299	2.2007	1.1581	1.7695	2.2066	2.6262	2.5461	2.4802	2.8638	1.3811	1.8499	0.4242		NS	NS					NS	NS	NS	NS								
50-a	2.5604	3.3261	3.9111	3.5815	2.5163	2.4935	2.1781	2.5868	2.9318	2.8846	2.0611	2.9210	1.8414	2.0986	0.7728	0.5868		NS					NS	NS	NS									
50-b	2.4887	3.2981	3.8589	3.4136	2.1961	2.1716	1.9508	2.3100	2.5399	2.5470	1.8784	2.6839	1.8493	1.8196	0.8988	0.8825	0.6055						NS	NS						NS				
51-a	2.0882	2.4892	3.2827	2.6386	1.6770	1.7460	2.1251	2.0647	1.8016	1.5295	2.9570	3.3389	2.9639	2.6198	3.1313	3.2590	3.4743	3.1141		NS														
51-b	2.2017	2.0774	3.3587	2.7033	1.7486	1.8026	2.1973	2.0752	1.7856	1.5256	3.1149	3.4527	3.0616	2.7108	3.3015	3.4330	3.6797	3.3170	0.3188															
52-a	1.5808	2.3445	3.3669	2.8981	0.9434	0.9425	0.9766	1.2432	1.4900	1.1779	2.0418	2.7360	1.3422	1.5880	1.6582	1.8006	2.2094	2.0651	1.7746	1.8881		NS				NS	NS	NS	NS	NS				
52-b	1.0360	1.5420	2.2674	1.6947	1.9380	1.9789	2.1030	2.3104	2.2795	2.1273	2.2180	2.9887	2.4197	2.4254	2.2380	2.2570	2.4054	2.1575	1.6411	1.8394	1.5418					NS								
53-a	3.3460	4.0564	4.9055	4.5182	2.4164	2.4552	2.3858	2.7626	2.8824	2.9032	1.9598	2.5453	1.8312	1.9049	1.2934	1.5210	1.3908	1.4767	3.6265	3.8419	2.3885	2.8242		NS										
53-b	3.5567	4.1467	4.9417	4.5596	2.8037	2.8935	2.9627	3.3230	3.2694	3.3570	1.9521	2.3793	2.3736	2.2399	1.7627	1.9744	1.6708	1.6955	3.8016	4.0453	3.0204	3.1029	0.8490			NS	NS	NS						
54-a	1.9436	2.3932	3.4274	3.2334	2.2466	2.2706	2.1623	2.5671	2.8315	2.5760	2.2663	3.2086	1.8149	2.5405	1.7790	1.7978	2.0849	2.2503	2.8090	3.0042	1.5002	1.2035	2.2689	2.1259		NS					NS	NS		
54-b	2.3171	2.8866	4.0052	3.7051	1.7775	1.8112	1.7324	2.0855	2.3850	2.0493	2.4145	3.1430	1.6809	2.2205	1.9091	2.0365	2.3721	2.4296	2.5282	2.6978	1.9479	2.2952	2.6119	2.6177	1.0245						NS	NS		
56-a	3.7485	4.0264	4.7693	4.3253	3.1754	3.3430	3.7686	4.0505	3.5438	3.8127	1.7351	1.7653	3.1071	2.7298	2.8037	3.0662	2.8379	2.6710	3.7433	3.9733	3.3378	3.0440	2.4983	1.8430	3.2319	3.4256		NS						
56-b	3.1623	3.5781	4.5529	4.0423	2.0310	2.2349	2.7693	2.9869	2.4699	2.6637	1.4211	1.4781	2.3223	1.8274	2.3072	2.6417	2.6129	2.3831	2.7277	2.9418	2.3135	2.5175	1.9960	1.7332	2.5671	2.3761	1.4295							
57-a	2.3881	3.2372	3.9342	3.3149	1.2133	1.1467	1.1003	1.2221	1.3953	1.1894	2.3885	2.9364	2.1291	1.7083	1.9501	2.0701	2.1890	1.7449	1.8230	1.9869	1.4915	1.9186	2.2961	2.6505	2.4842	2.1304	3.3042	2.3586		NS				
57-b	2.2792	3.1210	3.7691	3.3350	1.9445	1.8832	1.3999	1.7276	2.3190	1.9268	2.8170	3.5457	2.3596	2.3201	1.8741	1.8171	1.8766	1.6524	2.3347	2.5482	1.6934	2.0533	2.1577	2.5565	2.1507	1.9077	3.5644	2.7334	1.0684					
58-a	1.5999	1.6459	2.7917	2.5484	2.3320	2.3622	2.5081	2.6713	2.7166	2.4404	2.6830	3.6073	2.5436	3.0359	2.9191	2.9752	3.3571	3.3216	2.1754	2.2344	1.6744	1.7891	1.6750	3.9614	1.7192	1.9671	4.0024	3.2113	2.9064	2.9499		NS	NS	NS
58-b	2.2176	2.1824	3.3693	3.1786	2.5913	2.6509	2.8869	3.0743	3.0160	2.8099	2.8985	3.5726	2.6154	3.2005	3.1026	3.2094	3.5860	3.6057	2.6490	2.7187	2.0084	2.3046	3.7033	3.9733	1.7358	1.9685	3.9624	3.1857	3.2977	3.3424	0.7357			NS
60-a	2.5024	2.3898	3.5394	3.2143	2.5328	2.6689	3.1833	3.3421	2.9160	2.9345	2.3661	2.8281	2.6912	2.9716	3.1637	3.3660	3.6450	3.5706	2.5843	2.6721	2.2613	2.2371	3.6493	3.7155	2.1938	2.4216	3.2115	2.5922	3.3383	3.6109	1.4789	1.1696		NS
60-b	3.0207	2.8372	3.9708	3.6638	2.9189	3.0490	3.6053	3.7045	3.2277	3.2469	2.9394	3.2648	3.1620	3.4345	3.7617	3.9760	4.2912	4.2082	2.8775	2.9073	2.7183	2.8191	4.2158	4.3160	2.7363	2.8375	3.7802	3.0949	3.8155	4.1391	1.7685	1.3510	0.7039	
71-a	2.9681	3.2766	4.0759	3.8376	3.0066	3.0826	3.1193	3.5089	3.5499	3.3714	2.6775	3.4825	3.0117	3.2382	2.5692	2.6094	2.4690	2.4905	3.1954	3.4904	2.6531	2.4432	2.3207	2.2212	1.8016	2.1334	2.6519	2.3414	2.7573	2.3495	2.9816	2.9824	2.9822	3.5479
71-b	3.2771	3.5756	4.4188	4.2419	3.2804	3.3593	3.3216	3.7202	3.8606	3.6162	3.1016	3.8424	3.2542	3.5121	2.8283	2.8536	2.7303	2.8234	3.4817	3.7740	2.4186	2.3665	2.8877	2.8889	1.9869	2.1509	3.0263	2.6018	3.0289	2.4845	3.2488	3.2056	3.3165	3.8357
73-a	3.1096	3.7062	4.5502	4.0191	1.9610	2.0003	2.2166	2.4071	2.2788	2.1006	2.6826	3.2191	2.7648	2.5465	2.6520	2.8193	2.8273	2.5123	2.1968	2.4387	2.1497	2.4293	2.4125	2.6160	2.4467	2.0433	3.1236	2.1472	1.5453	1.7328	3.0482	3.2083	3.1870	3.6002
73-b	3.1492	3.7062	4.6716	4.1509	1.7928	1.8573	2.1772	2.3265	2.1366	1.9336	2.7143	3.1685	2.6674	2.4881	2.7724	2.9810	3.0855	2.8140	2.0908	2.2962	2.0318	2.5602	2.5518	2.8205	2.4027	1.8147	2.2831	2.1189	1.7323	1.9940	2.8497	2.9320	2.9441	3.2747
74-a	1.9698	2.6770	3.4410	2.7976	1.1511	1.1051	1.3732	1.4407	1.3208	1.0817	2.3500	2.9874	2.2173	2.0376	2.2867	2.4080	2.6174	2.2349	1.2125	1.3692	1.2545	1.3784	2.8271	3.1281	2.2149	2.0233	3.3892	2.4609	1.0209	1.6238	2.1421	2.5747	2.6267	3.0522
74-b	2.0271	2.6404	3.2736	2.5667	1.5083	1.5246	1.9019	2.0000	1.6265	1.6825	2.0037	2.6328	2.3642	2.0454	2.2774	2.4133	2.5060	2.0528	1.5280	1.7062	1.6829	1.1454	2.8430	2.9568	2.4297	2.4826	2.8889	2.2553	1.3505	1.9845	2.4153	2.8239	2.5940	3.1005
75A-a	2.9983	3.7304	4.3324	3.7876	2.2034	2.1141	2.0502	2.2418	2.3531	2.1782	2.8702	3.6140	2.7681	2.7208	2.4997	2.5505	2.5492	2.1976	2.5464	2.7430	2.2799	2.3737	2.6096	2.9428	2.5668	2.4462	3.6018	2.9109	1.4578	1.5780	3.1832	3.4776	3.6171	4.0774
75A-b	2.6926	3.4243	4.1245	3.6953	2.1670	2.0867	1.8240	2.1270	2.4960	2.1602	2.8954	3.7231	2.5348	2.7065	2.2358	2.2322	2.2939	2.1018	2.5488	2.7647	1.9609	2.2743	2.3520	2.7793	2.0133	1.8586	3.6680	2.8806	1.5191	1.1624	2.8463	3.1175	3.4430	3.9169
76A-a	3.0198	3.6088	4.4590	3.8591	1.6748	1.6664	2.1190	2.2225	1.8056	1.8256	2.3490	2.9244	2.4468	2.3478	2.6337	2.8493	2.9811	2.6380	2.1524	2.3046	1.9936	2.3032	2.7326	3.0177	2.4339	2.1829	3.2365	2.3462	1.7286	2.2717	2.6795	2.8043	2.7274	3.0725
76A-b	2.9381	3.3337	4.2927	3.7530	1.8999	1.9277	2.4289	2.4854	2.0715	1.9625	2.7629	3.2905	2.7559	2.8000	3.1415	3.3426	3.5712	3.3029	1.9160	2.0168	2.0309	2.3553	3.3578	3.6404	2.4274	2.1460	3.6742	2.6791	2.2851	2.7270	2.1459	2.2004	2.2222	2.4009
77-a	2.8759	3.0505	3.4765	2.9810	3.0528	3.1883	3.4838	3.6841	3.3633	3.3945	2.6975	3.0413	3.6448	3.1772	3.1818	3.2731	3.0647	2.7292	2.6325	2.8980	3.0748	2.0641	3.3013	2.8830	3.2546	3.4634	2.1867	2.2374	2.7254	2.6806	3.5438	3.8306	3.2964	3.8724
77-b	3.1090	3.2811	3.8650	3.3167	2.8825	3.0500	3.5005	3.6619	3.1665	3.2723	2.5323	2.6967	3.5847	3.0211	3.2853	3.4557	3.2979	2.9354	2.5148	2.7640	3.0516	2.2444	3.3020	2.8802	3.3344	3.4212	1.9614	1.8746	2.7368	3.0645	3.5345	3.7442	3.0773	3.5958
78-a	2.7809	3.2782	3.9706	3.4152	2.1619	2.2473	2.4912	2.6856	2.4809	2.3650	2.5677	3.0731	3.0179	2.6262	2.6963	2.8236	2.7231	2.3361	1.9543	2.2499	2.2944	1.9511	2.6192	2.5558	2.6209	2.4819	2.6776	1.9647	1.5979	1.7881	3.0887	3.3565	3.1431	3.6469
78-b	2.8166	3.1648	4.0659	3.5665	2.1176	2.2611	2.6269	2.7944	2.5212	2.3447	2.7027	3.0924	3.0633	2.7348	2.9848	3.1586	3.1743	2.8809	1.7261	2.0120	2.2043	2.1190	2.8737	2.8449	2.5244	2.2141	2.8416	1.8425	1.9924	2.1742	2.7678	2.9303	2.7052	3.1112
78A-a	2.7466	3.4011	4.3036	3.7459	1.3789	1.4412	1.7232	1.6991	1.7555	1.5697	2.3429	2.8378	2.3104	2.0049	2.2900	2.4921	2.5786	2.2468	1.8321	2.0578	1.6440	2.1410	2.2023	2.4676	2.2478	1.7372	2.9936	1.8565	1.1182	1.4983	2.7636	2.9594	2.9273	3.3524
78A-b	2.9717	3.5454	4.5296	4.0434	1.7218	1.8174	2.0642	2.2838	2.1841	1.9752	2.4890	2.9529	2.4512	2.2537	2.4474	2.6562	2.7309	2.4986	2.1514	2.3865	1.8544	2.4322	2.1491	2.3647	2.1815	1.5818	2.9748	1.7858	1.6239	1.7471	2.8433	2.9289	2.9168	3.3205
81-a	2.7828	3.2151	3.9427	3.4235	2.2879	2.4141	2.5849	2.6439	2.5865	2.3353	3.2392	3.4504	3.3636	2.7787	3.1981	3.3149	3.3485	3.0176	1.7014	1.8083	2.4125	2.3869	3.2710	3.2724	3.2138	2.7953	3.4431	2.4472	1.9829	2.1662	3.3002	3.6588	3.5095	3.9119
81-b	3.0866	3.5085	4.1290	3.5583	2.5350	2.6476	2.8513	2.8304	2.7038	2.5017	3.5569	3.6741	3.7200	3.0247	3.5766	3.6954	3.7232	3.3382	1.9059	1.9538	2.7744	2.6748	3.6855	3.6751	3.7034	3.2803	3.7459	2.7996	2.1967	2.5155	3.6549	4.0527	3.8572	4.2245
82-a	3.3313	3.6468	4.4605	4.1597	3.1063	3.1753	3.0976	3.1534	3.4564	2.9362	4.3994	4.7473	4.0407	3.8369	4.0294	4.0772	4.2339	4.0719	2.4292	2.5395	2.9293	3.3063	4.0672	4.2843	3.4735	2.8616	4.7934	3.6327	2.9271	2.7065	3.4324	3.7243	4.0143	4.2514
82-b	3.3862	3.6433	4.2458	3.9592	3.4797	3.5414	3.4295	3.4704	3.7769	3.2770	4.7063	5.0616	4.4661	4.1832	4.3268	4.3314	4.4318	4.2316	2.5499	2.6617	3.3000	3.3555	4.4467	4.5841	3.8700	3.4095	4.9888	3.9671	3.1261	2.8853	3.7544	4.1470	4.3786	4.6568
83-a	1.5790	2.1316	2.5770	2.1619	2.4423	2.4378	2.4174	2.6750	2.7247	2.7696	1.9240	2.7902	2.0898	2.3241	1.7435	1.6956	1.8503	1.7636	2.9204	3.0535	2.0550	1.4852	2.8506	2.9442	2.2173	2.7878	3.0102	2.8918	2.5281	2.6243	2.5114	2.8869	2.7829	3.4101
83-b	1.5139	2.1878	2.4894	1.9875	2.3918	2.3557	2.2558	2.4713	2.5987	2.6071	2.2050	3.0228	2.2592	2.3658	1.8649	1.7768	1.9169	1.7260	2.7568	2.8775	2.0565	1.4141	2.9851	3.1199	2.4381	2.9088	3.2708	2.0684	2.3052	2.4291	2.6399	3.1135	3.0714	3.6755
84-a	1.7826	2.0915	2.7379	1.9984	1.8960	1.9905	2.4486	2.4910	2.0280	2.1094	2.2105	2.6310	2.7198	2.3591	2.7551	2.8904	3.0330	2.6602	1.4205	1.5290	1.9516	1.0635	3.4500	3.4475	2.7480	2.8734	2.9784	2.4305	2.1316	2.6830	2.2469	2.6933	2.2546	2.7275
84-b	2.3063	2.4326	2.8405	2.4910	2.6065	2.7106	3.1395	3.2319	2.7512	2.8628	2.4777	2.8673	3.3284	2.9322	3.1656	3.2772	3.2710	2.8837	2.0134	2.1777	2.6636	1.4380	3.7086	3.5229	3.1463	3.4075	2.7454	2.5674	2.5982	3.0497	2.8308	3.2154	2.6361	3.1398

TABLE 8 (cont.): The matrix of values of the Mahalanobis Distances between each group pair below the diagonal, with the significance of each distance above the diagonal (where NS = not significant, otherwise $p < 0.05$).

OTU	71-a	71-b	73-a	73-b	74-a	74-b	75A-a	75A-b	76A-a	76A-b	77-a	77-b	78-a	78-b	78A-a	78A-b	81-a	81-b	82-a	82-b	83-a	83-b	84-a	84-b
1-a																								
1-b						NS				NS														
3-a																								
3-b					NS							NS	NS										NS	
4-a					NS	NS			NS	NS					NS								NS	
4-b					NS	NS			NS	NS													NS	
9-a					NS	NS			NS	NS					NS	NS								
9-h					NS	NS			NS	NS					NS									
14A-a					NS	NS			NS	NS														
14A-b					NS	NS			NS	NS														
16-a			NS	NS					NS	NS			NS	NS	NS								NS	
16-b					NS	NS			NS	NS			NS	NS	NS									
16-e					NS	NS			NS	NS					NS								NS	
18-b					NS	NS			NS	NS													NS	
20-a					NS	NS			NS	NS														
20-b					NS	NS			NS	NS														
20A-a						NS															NS	NS		
20A-b																					NS	NS		
23-a									NS	NS														
23-b									NS	NS														
24-a					NS	NS			NS	NS													NS	
24-b					NS	NS			NS	NS													NS	
27A-a																							NS	
27A-b																							NS	
28A-a																								
28A-b																								
28B-a																								
28B-b																								
31-a																								
31-b																								
32-a																								
32-b																								
34-a									NS	NS														
34-b																								
35-a																					NS	NS		
35-b																								
38A-a																								
38A-b																								
39-a					NS	NS			NS	NS					NS									
39-b					NS	NS			NS	NS					NS									
41-a					NS																			
41-b					NS																			
43-a					NS	NS									NS									
43-b					NS	NS			NS	NS					NS									
45-a						NS			NS						NS						NS	NS	NS	
45-b																								
47-a																								
47-b																								
48-a																								
48-b																								
50-a																								
50-b																								
51-a					NS	NS				NS													NS	
51-b					NS					NS													NS	
52-a					NS																			
52-b					NS	NS															NS	NS	NS	NS
53-a																								
53-b				NS																				
54-a	NS	NS	NS	NS				NS																
54-b	NS				NS			NS	NS	NS					NS	NS								
56-a																								
56-b																								
57-a			NS		NS	NS	NS	NS	NS				NS		NS	NS								
57-b					NS		NS	NS							NS									
58-a																								
58-b																								
60-a																								
60-b																								
71-a		NS																						
71-b	0.6663																							
73-a	1.9744	2.1813		NS			NS	NS	NS	NS			NS	NS	NS	NS								
73-b	2.2149	2.3453	0.6164						NS	NS			NS	NS	NS	NS								
74-a	2.6644	3.0340	1.6053	1.6570		NS	NS		NS	NS			NS		NS								NS	
74-b	2.6324	3.1247	1.8874	2.0842	0.8263				NS	NS			NS		NS								NS	NS
75A-a	2.3743	2.7197	1.2689	1.7016	1.5095	1.7378		NS	NS				NS		NS									
75A-b	2.0027	2.2180	1.2852	1.5998	1.5981	2.0033	0.8189		NS						NS	NS								
76A-a	2.4908	2.8747	1.2010	1.2155	1.3060	1.5352	1.3228	1.6237		NS			NS	NS	NS	NS								
76A-b	2.7320	3.0512	1.7035	1.4317	1.5378	1.9174	2.0676	2.1231	1.0003				NS		NS	NS								
77-a	2.5199	2.9335	2.7156	3.0177	2.6634	2.1291	3.0818	3.1780	3.0850	3.3706		NS		NS										NS
77-b	2.6322	3.0330	2.5828	2.7984	2.6294	2.1165	3.1378	3.2807	2.6880	3.1303	0.6768													NS
78-a	1.9041	2.2385	1.0733	1.4815	1.6231	1.5352	1.6741	1.7803	1.7713	2.1962	1.6962	1.6595		NS	NS	NS								
78-b	1.9992	2.1879	1.2296	1.2292	1.8068	1.9287	2.2467	2.1520	1.9002	1.9270	2.0418	1.8202	0.9437		NS	NS								
78A-a	2.1675	2.3725	0.6383	0.6701	1.2568	1.6261	1.4714	1.4077	1.2349	1.6746	2.6306	2.4779	1.1512	1.1838		NS								
78A-b	1.9627	2.0398	0.7560	0.5653	1.7394	2.0964	1.8475	1.6111	1.5508	1.8236	2.7781	2.5957	1.3666	1.1078	0.5797									
81-a	3.0667	3.1523	2.2327	2.2499	2.1897	2.3494	2.9397	2.8143	2.9128	3.0028	2.2505	2.1833	1.7340	1.5325	1.9914	1.8893		NS						
81-b	3.5560	3.6736	2.5658	2.5978	2.4114	2.5287	3.1915	3.1714	3.1699	3.2721	2.4381	2.3506	2.0340	1.9204	2.4127	2.2359	0.5642							
82-a	3.5586	3.3849	2.8286	2.6380	2.9717	3.4968	3.5663	3.1526	3.5804	3.3461	3.7169	3.6901	2.8393	2.3318	2.6174	2.4910	1.7888	2.0425		NS				
82-b	3.8238	3.7053	3.1747	3.1124	3.1820	3.6012	3.7800	3.4399	3.9431	3.7656	3.5858	3.6506	2.9427	2.5911	2.9918	2.9614	1.7643	1.8814	0.8296					
83-a	3.0229	3.4904	3.3360	3.5029	2.3348	1.9617	3.0018	2.9249	3.0347	3.3191	2.7874	3.0212	2.9677	3.2954	3.0064	3.2950	3.4612	3.7389	4.4839	4.5697		NS		
83-b	3.1763	3.6389	3.2754	3.4803	2.1602	1.8029	2.8319	2.8081	3.0037	3.3305	2.7721	3.0458	2.8757	3.2828	2.9428	3.2979	3.3140	3.5407	4.3586	4.3858	0.5320			
84-a	3.1316	3.5948	2.7074	2.7634	1.5689	1.1179	2.8122	2.9309	2.4151	2.4301	2.0253	1.9777	2.1528	2.2341	2.3498	2.7026	2.3444	2.4661	3.5623	3.5849	1.9498	1.8649		NS
84-b	3.0760	3.5985	2.9122	3.0913	2.1239	1.4647	3.0169	3.2208	2.7370	2.8431	1.4644	1.4928	2.1219	2.3497	2.7067	3.0210	2.6000	2.6951	3.9580	3.8763	2.2355	2.1772	0.9022	

TABLE 8 (cont.): The matrix of values of the Mahalanobis Distances between each group pair below the diagonal, with the significance of each distance above the diagonal (where NS = not significant, otherwise $p < 0.05$).

separations in this group. The only significant differences between any two of these OTUs were found for **20A** between **9, 14A, 16** and **23**. Interestingly, the OTUs **18** and **20** were nonsignificantly different to **20A**.

OTUs **27A, 28A, 28B** and **31**

No evidence was provided by this analysis to separate this group; in all cases the distances between and within OTUs were not significant.

OTUs **32** and **34**

Whilst these two OTUs showed closer morphometric affinities to each other than they did to any other OTU (Table 8), the distances between them are significant and as such warrant their recognition as discrete taxa.

OTUs **35** and **38A**

These two OTUs are distinguishable from all the remaining OTUs in the analysis, but not significantly different to each other, and this information substantiates their inclusion in one species group.

OTUs **39, 41** and **43**

These three OTUs were indistinguishable from each other morphometrically; hence the specific status of this group must remain in doubt.

OTUs **45, 47, 48** and **50**

The OTU **45** showed the same pattern of variation when compared with any of the other three OTUs in this group, where the males were not significantly different to both males and females of the opposing OTU, but females always exhibited significantly large Mahalanobis Distances. One possible explanation for this pattern is the sample size for the males of **45** (n = 3; compared to the female sample size of n = 10), since the tests for significance are closely dependent upon the sample sizes of the two groups being tested. Nevertheless, the consistent separations between females in the OTU **45** and the other OTUs strongly suggests that this OTU can be retained as a discrete species. The relationships between **47, 48** and **50** are interesting in that the sympatric **47** and **50** are clearly separated by significant D-values whilst **48** and **50** are not significantly different; the evidence for **47** and **48**, however, is somewhat inconclusive with males of **47** being different to both forms of **48**, but females not. This suggests that morphometrically, **48** is intermediary to **47** and **50**.

OTUs **58** and **60**

The nonsignificant differences between these two OTUs, and their significant distances to the remainder of the OTUs in this study equate with the electrophoretic results for these OTUs; no extra information is therefore provided to distinguish between these two OTUs.

TASMANIAN AND VICTORIAN OTUs

Of the 32 OTUs from Victoria, **1, 32** and **34** each showed significant differences to every other OTU in the study; in addition, the OTUs **58** and **60** were not significantly different to each other but both showed significant differences to all other OTUs.

Of the 14 OTUs from Tasmania, **71, 77, 81** and **82** each showed significant differences to every other OTU in the study.

On all other occasions, each OTU was found to be not significantly different from at least one other OTU.

One could hypothesize that if Tasmanian OTUs are morphometrically more closely related to themselves than they are to Victorian OTUs, then one would expect that the proportion of the number of nonsignificant differences within the OTUs from Tasmania would exceed the proportion of nonsignificant differences found between Tasmanian OTUs and Victorian OTUs. To test this, the Tasmanian OTUs were selected (excluding **14A** since specimens of this morphology can be found in Victoria, and **71**, **77**, **81** and **82** as above) and for each OTU two values were calculated

i) the sum of the number of nonsignificant differences found between it and other Tasmanian OTUs, divided by the total number of comparisons made (ie. 308), and

ii) the sum of the number of nonsignificant differences found between it and Victorian OTUs, divided by the total number of comparisons made (2112).

These values are presented in Table 9 and whilst statistical comparisons are not valid due to a lack of independence of these values, they suggest indeed that the majority of Tasmanian OTUs are morphometrically more closely related to each other than they are to Victorian forms.

	N_{Tas}	P_{Tas}	N_{Vic}	P_{Vic}
51	7	0.0227	9	0.0043
73	14	0.0454	6	0.0028
74	15	0.0487	47	0.0223
75A	9	0.0292	6	0.0028
76A	19	0.0617	45	0.0213
78	15	0.0487	6	0.0028
78A	17	0.0552	17	0.0080
83	0	0.0000	10	0.0047
84	7	0.0227	13	0.0062

TABLE 9: The Tasmanian OTUs, the number and proportion of nonsignificant differences found between each other (N_{Tas} and P_{Tas}) and the number and proportion of nonsignificant differences found between themselves and the Victorian OTUs (N_{Vic} and P_{Vic}) from Table 8.

Discussion

CHOICE OF VARIABLES

In morphometric analyses it seems that much disagreement occurs over the conditions which govern the choice of, and pre-analysis treatment of, the variables. For instance it might seem intuitively logical to include in a multivariate analysis as many variables describing as much morphological variation as possible. Some authors (for instance Humphries et al., 1981) suggest that the treatment of measured or continuous variables be separated from meristic or discontinuous variables since the latter impose different statistical problems.

Each morphological measurement includes a shape component, or the characteristics of that measurement which are peculiar to the organism, plus a size component. Since the organisms in a morphometric study are never likely to be all the same age, the shape components need to be independent of size in order to partition out the effects of age-related growth (Humphries et al., 1981). Whilst admitting that "...size-related shape effects are inextricably mixed-up in almost every biological feature of organisms which we may wish to investigate...", Oxnard (1978) suggests that one useful way of dealing with size effects is by the use of ratios since they eliminate the portion of size that relate to isometry (even though ratios do not completely remove the effects of size, Gould, 1966). However, Atchley et al., 1976, and others have highlighted the difficulties involved in the use of ratios (summarised in Humphries et al., 1981). Blackith and Reyment (1971) and Dodson (1978) consider that these problems can largely be avoided by a log-transformation of each ratio. Humphries et al. (1981) and Mosimann and James (1979) disagree with this interpretation, since, amongst other objections, they consider that not all ratios are log-normally distributed.

In morphometric analyses, therefore, it seems that the choice of variables and their pre-analysis treatment should be approached with caution. The above arguments have largely been heeded in this study by the selection of only measured distances (or continuous variables), the subsequent elimination of 49 of the 55 initial measured variables to give a final 6 variables which conformed to a number of biological and statistical properties, including a normal distribution following the log-transformation of each ratio.

CHOICE OF ANALYTICAL TECHNIQUE

Morgan (1983), in his morphometrical treatment of the parastacid genus *Euastacus*, chose an alternative approach, primarily in the selection of a polythetic agglomerative program (a multiple classificatory system) which did not require the prior selection of groups (as is required for discriminatory analyses such as canonical variate analysis), and also in the use of 108 variables of mainly a meristic or a discontinuous nature but including 7 continuous variables in the form of ratios. His results showed separations between groups of crayfish individuals; in addition, individuals of some species (discerned in retrospect) turned up in miscellaneous groups and each 'misfit' could be interpreted as being the result of either allometric effects (since the effects of size were deliberately included in the analysis) or interpopulational variation over a geographical range.

On the other hand, Sumner (1978) used a similar multivariate approach with 27 variables, on the freshwater crayfish *Parastacoides*. Whilst the author claimed to include only isometric variables, many of his characters were likely to show allometric effects (Morgan, 1983). In addition, Sumner's truncated phenogram revealed at least 5 major clusters of individuals at his '...first level of major phenon formation...'; however it seems that on the basis of a failure of the analysis to provide diagnostic characters for all five groups, the hasty claim was made that only one species (comprising three subspecies) of *Parastacoides* existed. Closer examination of his results suggest that individuals in each of the five groups could have been diagnosed at least by using combinations of characters.

The approach taken in this study was merely utilitarian, to search for further evidence with which to separate problem groups of populations which had been partially identified by a previous technique (electrophoresis). The canonical variate analysis turned out to be adequate for this purpose. The consistent non-discrimination of males and females from the same population, and the non-discrimination of sub-groups from the same population, assured at least some biological validity to the degrees of separations expressed between OTUs. A minor flaw in the technique was exhibited with the non-discrimination of already discriminated OTUs from the electrophoretic technique; since adequate evidence for the separation of these OTUs from others was previously derived, this short-coming was not considered to be important.

The failure of the analysis to provide an easily interpreted graphical representation of the results, through the recognition of six canonical variates which each contributed a significant proportion of variation to the overall analysis, is interesting since the results almost certainly reflect the amount of morphological variability in the genus *Engaeus* where no one or two measured variables could be used to explain a large amount of the morphological variation. Much of the variation might be related to environmental factors; in fact any further investigation into the morphological proportions of these freshwater crayfish could be productive if the habitat of each population could be incorporated into the analysis. For instance in Figure 2 the univariate analysis provided for each variable exhibited consistent separations between populations which occupy type 1 burrow habitats and those which are capable of occupying type 3 burrow habitats (sensu Horwitz and Richardson, 1986). OTUs **32, 34** and **71**, which are commonly found in type 1 habitats, frequently appear at one end of the linear spread whilst OTUs **27A, 28A** and **28B** (found in type 3 burrow habitats) appear at the opposite end. Of course the spread of OTUs between these two groups was not consistent; nevertheless, such an incorporation of habitat types into a multivariate analysis might be productive in reducing the number of variables which describe significant amounts of variation to a number which can be interpreted qualitatively without having to cope with 6-dimensional problems.

The similarity within most of the Tasmanian OTUs when compared to Victorian OTUs can be interpreted in two ways. Either these Tasmanian OTUs are phylogenetically more closely related to each other than they are to the Victorian OTUs, with the genetical

affinities being translated into morphological affinities, or the environmental conditions in Tasmania have combined to produce morphologically similar body shapes from phylogenetically diverse OTUs. Obviously further information will have to be assessed before one or other of these explanations can be hypothesized.

No evidence was provided to separate the OTU pair of **58** and **60**, nor **35** and **38A**, into discrete taxa; the OTUs **27, 28** and **31** are considered to be conspecific, and the OTU group **39, 41** and **43**, whilst comprising at least 2 species according to electrophoretic data, could not be morphometrically distinguished.

Contrary to the electrophoretic evidence which tentatively suggested the lumping of OTUs **32** and **34**, information provided in this analysis suggested that despite being more similar to each other than they are to all other OTUs, they should be treated as separate species. Further information needs to be assessed to resolve this question. The OTUs **73** and **75A** could be morphometrically separated, thus substantiating the ideas presented in electrophoretic section.

The OTU **45** was successfully separated from OTUs **47, 48** and **50**. The electrophoretic study found strong evidence to combine the OTUs **47** and **48**, and to separate **50**; the analysis here presents a somewhat different story, however, with **48** apparently intermediary between **47** and **50**. This has an ecological corollary since in sympatry **47** and **50** occupy different microhabitats (Horwitz et al., 1985b) and are subject to different environmental constraints, whilst individuals from **48** occupy the entire range of microhabitats.

Finally, very little information on the status of the OTUs **9, 14A, 16, 18, 20** and **20A** could be elucidated from the canonical variate analysis. The possible grouping of **18, 20** and **20A** received support since no other OTU in the group showed insignificant differences to **20A**. **20A** is the most eastward OTU in this group and the results here may suggest a morphological cline in an easterly direction.

In summary therefore the technique of canonical variate analysis as used in this work provided some valuable data on the delineation of species in the overall analysis and an interesting comparison between the morphometrical attributes of Tasmanian and Victorian OTUs. However, the failure of the technique to separate between morphologically and electrophoretically diverse OTUs such as **4** and **51**, or **54** and **28B**, surely casts some doubts on the use of this technique to discriminate between OTUs in the absence of another independent technique such as electrophoresis.

Section 2.4 FINAL DELINEATION OF SPECIES

Introduction

The evidence presented in this chapter thus far has provided sufficient information to clearly delineate most of the OTUs into species. However, the techniques used have failed to delineate species from within a few groups of OTUs where between OTU distances are inconclusive (ie. defining neither conspecific nor congeneric OTUs). These results have been produced without the use of classical taxonomic techniques such as the examination of external morphological features.

The aim of this section is twofold. Firstly, the external morphology of individuals of those difficult OTUs have been examined for reliable characters which can consistently separate between the OTUs. If such characters could be found, then the OTUs were considered to be distinct species; if not, the OTUs were combined into one species.

Secondly, the data in this chapter were summarized to produce a final delineation of the proposed species in the genus *Engaeus* and the species were equated to previously described taxa where applicable. This final discussion includes the significant morphological features which can be used to identify each species.

The morphological characters outlined in this section are discussed in more detail in Chapter 5. The information used in this section contributes to the taxonomic key to the genus *Engaeus* which is presented in Chapter 6 (Section 6.3).

Delineation of Undiscriminated Taxa

THE OTU GROUP **6-26**

The individuals in this group are all morphologically similar, each with a multiarticulate exopodite of the third maxilliped and (in almost all cases) a full complement of sternal pores. Most of the valuable variation within the group occurs in characters such as the setation, granulation and tuberculation of the chelae.

Individuals in the OTUs **21-26** can be immediately distinguished from those in the remaining OTUs by the presence of a thick pad of plumose setae over the ventral half of ONLY the mesal surface of the propodal palm. OTUs **6-20** either exhibit such a pad over BOTH the mesal and the lateral surfaces of the propodal palm, or exhibit a reduced level of plumose setation. In the OTUs **24-26**, individuals never exhibited plumose setation of the ventral surface of the carpus and the merus. **21** and **23**, on the other hand exhibited this distinctive setation; OTU **22** displays the former character state and as such presents a contrast to the electrophoretic results, where it is more closely linked to **21** and **23** than **24-26**. For practical convenience this anomalous OTU will be combined with the latter OTUs.

Individuals in OTU **16** display a sub-carinate row of small tubercles on the ventral surface of the propodal palm (displaced slightly laterally); this character distinguishes it from individuals in all other OTUs in this group.

The remaining OTUs can be split into two morphological groups, **6-15** and

17-20. The former group exhibits granulations over the dorsal surface of the dactyl of large dimorphic chelae and most isomorphic chelae; these granulations are absent on the chelae of individuals in the latter group.

The OTUs **17-20** (incorporating **20A**) are morphologically variable and much of this variation appears to have a geographical component; for instance for populations in the geographical region between the OTUs **20** and **20A**, individuals exhibit a gradual decrease in the setation of the propodal palm and an increase in the frequency of the absence of sternal pores on the lateral processes of the 1st and 2nd pereiopods. The non-significant differences exhibited between **20A, 20** and **18** in the morphometric analyses are here assumed to represent a morphological affinity, in distinction to the OTUs in the group **6-15**.

THE OTUs **32-34**

The results from the electrophoretic analysis indicate that there is some important variation between these three OTUs; similarly the morphometric results suggested that **32** and **34** could at least be considered as distinct taxa. Despite this evidence, these OTUs will be classed as comprising a single species since it is here considered that the patterns of variation for this species need to be more fully understood, with the incorporation of morphologically very similar individuals from Tasmania into any analyses before any further delineation, if warranted, can be made.

THE OTUs **39-44**

The only conclusive piece of evidence to suggest a possible break-up of this group comes from the electrophoretic recognition of two OTUs in sympatry (ie. **39** and **41**). These two OTUs are easily distinguished by the presence or absence (respectively) of granulations over the mesal and lateral surfaces of the propodal palm. There is no apparent morphological evidence to separate the most widely divergent OTU, **44**, from the remaining OTUs. Consequently, only two species will be recognised from this diverse group, namely those without the character described above (**40-44**), and that with the character.

Final Delineation of Species

It is proposed here that the following OTUs constitute viable species according to the definition of the species provided in Chapter 1:

1-2: These OTUs correspond exactly to *E. phyllocercus* Smith and Schuster 1913. This species is recognised by the absense of sternal pores and by the presence of a large terminal spine on the inner ramus of the uropod.

3: This species is new and will be coded *Engaeus* VRJ; it is distinguished by the absence of sternal pores, the form of the rostrum and the rostral carinae, and by the dorsal and ventral tuberculation of the propodal palm.

4-5: These individuals are characterized by the presence of all sternal pores, the dorsal tuberculation of the propodal palm, the reduced exopodite of the third maxilliped and the nature of the rostral tip. They correspond to *E. fultoni* Smith and Schuster 1913.

6-15: Individuals of this group can be identified from other closely related species by the

presence of dactyl granulations (see above), and distinguished from other species by the presence of all sternal pores and a multiarticulate exopodite of the third maxilliped. They correspond to the holotype of *E. cunicularius* (Erichson 1846).

17-20,20A: These individuals can be identified by the lack of granulations on the dactyl, otherwise concurring with the preceding species, and correspond to *E. quadrimanus* Clark 1936a and *E. marmoratus* Clark 1941. This study therefore recommends the synonymy of these two names, with the former having chronological priority.

21 and **23**: This new species will be coded *Engaeus* VS (see preceding section for morphological affinities).

22, 24-26: Individuals in these OTUs show morphological congruence with the type specimens of the species *E. sericatus* Clark 1936a (see preceding section).

27-31: Individuals belonging to this species can be readily separated from other species by the absence of sternal pores, the absence of a transverse suture on the outer ramus of the uropod and the presence of a long antennal scale; they correspond to the described species of *E. hemicirratulus* Smith and Schuster 1913 and *E. jumbunna* Riek 1969. The former name has chronological priority.

32-34: The morphological features of these individuals, which include a well developed postorbital ridge and a distinctive sternal keel, concur with the type specimen of *E. laevis* (Clark 1941).

35-38,38A: These individuals exhibit no tubercles on the ventral surface of the propodal palm, no sternal pores and usually no exopodite of the third maxilliped; they are identical to the described species *E. cymus* (Clark 1936a) and *E. parvulus* Riek 1951, and in synonymy the former name has chronological priority.

39: These individuals exhibit tubercles on the ventral surface of the propodal palm, no sternal pores and usually no exopodite of the third maxiiliped; they belong to an undescribed species, *Engaeus* VAFA.

40-44: These OTUs agree with the species *E. affinis* Smith and Schuster 1913 and are similar to OTU **39** except for the absence of granulations on the propodal palm.

45-46: These OTUs agree with *E. victoriensis* Smith and Schuster 1913 and are characterized by the absence of sternal pores and a smooth ventral surface of the propodal palm, a reduced posterior pleurobranch and a prominent antennal scale.

47-49: Individuals belonging to these OTUs correspond to *E. tuberculatus* Clark 1936a and *E. connectus* Riek 1969; in synonymy the former has chronological priority. It displays an absence of sternal pores, tubercles along the ventrolateral line of the propodal palm, a prominent antennal scale and a reduced posterior pleurobranch.

50: This OTU agrees with the species *E. urostrictus* Riek 1969, occurs in sympatry with *E. tuberculatus*, its closest extant relative, and can be distinguished from the latter by the nature of the spination on the outer ramus, the form of the rostrum and the size of the antennal scale.

51: This species is undescribed and will be coded *Engaeus* TA; it is easily distinguished by the absence of a transverse suture of the outer ramus of the uropod and a multiarticulate exopodite of

the third maxilliped.

52: The morphological features of this OTU concur with that of *E. orientalis* Clark 1941; individuals of this species lack sternal pores, exhibit a reduced exopodite of the third maxilliped, non-tuberculate ventral surface of the propodal palm and they are never intersexed.

53: Individuals in this OTU correspond to the species *E. sternalis* (Clark 1936a); they are characterized by the shape of the orbital peduncle, the reduced number of sternal pores and the reduced size of the exopodite of the third maxilliped.

54: This species is undescribed and will be coded *Engaeus* VSL; its diagnostic features include the absence of sternal pores, a multiarticulate exopodite of the third maxilliped and the presence of both male and female gonopores on adult individuals.

55-56: The diagnostic characters of this species include an almost completely smooth (non-tuberculate) propodus of the chelae and a reduced exopodite of the third maxilliped; individuals apparently correspond to the description of *E. strictifrons* (Clark 1936a) even though the type specimens for this species cannot be found.

57: This OTU represents the species *E. australis* Riek 1969 and individuals of this species can be recognised by the multiarticulate exopodite of the third maxilliped, the presence of both male and female gonopores and the nature of the rostrum and the rostral carinae.

58-62: Individuals in these OTUs belong to the species *E. lyelli* (Clark 1936a) and can be identified by the reduced number of sternal pores and the unique form of the sternal keel and the annulus ventralis.

71: This undescribed species will be coded *Engaeus* TJ; it can be identified by a reduction in the number of sternal pores, its usually small size, a multiarticulate exopodite of the third maxilliped and by the nature of the tuberculation and carinae of the propodus.

72-73: The presence of sternal pores on the lateral processes of the 4th pereiopods, the presence of a multiarticulate exopodite of the third maxilliped and the occurrence of dense granulations over the propodal palm characterize this new species, to be coded *Engaeus* TBZ.

74: Individuals of this undescribed species can be immediately distinguished by the presence of sternal pores and the presence of a large terminal spine on at least the inner ramus of the uropod; it will be coded *Engaeus* TF.

75, 75A: This group represents another undescribed species, coded *Engaeus* TB, which is similar to *Engaeus* TBZ but distinguished by the absence of the propodal palm granulations.

76, 76A: Individuals belonging to these OTUs are without sternal pores, with a multiarticulate exopodite of the third maxilliped, and with a non-tuberculate propodal palm: they are undescribed and will be coded *Engaeus* TM.

77: This species is also undescribed and will be coded *Engaeus* TQ; individuals in this species lack sternal pores, exhibit a reduced exopodite of the third maxilliped and show large granulations over the propodal palm.

78-79, 78A: These individuals are recognised by a double row of tubercles on the dorsal surface, and a single row of tubercles on the ventral surface of the propodus, the presence of sternal pores and by their blunt rostra; they belong to *E. fossor* (Erichson 1846), the genotype of

Engaeus.

80-81: This group agrees with *E. cisternarius* Suter 1977a, a distinct species which can be recognised by the absence of a transverse suture on the outer ramus of the uropod and by the presence of sternal pores.

82: These individuals are charaterized by an absence of sternal pores, an absence of the transverse suture of the outer ramus of the uropod and by a reduced antennal scale; they belong to a new species, *Engaeus* TN.

83: Another new species, *Engaeus* TD, can be identified by the tuberculation of the propodus, the presence of sternal pores and the reduced exopodite of the third maxilliped.

84: This OTU belongs to the species *E. leptorhynchus* Clark 1939 and can be identified by the lack of sternal pores, the reduced exopodite of the third maxilliped, the form of the rostral region and the smooth ventral surface of the propodal palm.

Section 2.5 CONCLUSIONS

From the initial 78 OTUs presented in Section 2.1, 34 species in the genus *Engaeus* have been delineated. No evidence of hybridization between species was found, but evidence from both morphological and electrophoretic sources suggest that clines may exist for at least a few species of *Engaeus*.

In general, the three taxonomic techniques (allozyme electrophoresis, multivariate morphometrics and classical taxonomy) produced unambiguous results which supported the findings of each other. Only a few cases of ambiguity exist, for instance where a closely related group of OTUs exhibited substantial electrophoretic variation but this variation was not detected morphologically (for the OTUs **39-44**), or where an electrophoretic result suggested that an OTU should belong with one group, but the morphological results suggested otherwise (for the OTU **22**). Such ambiguities are much more likely to be the results of insufficient sampling rather than a product of inaccurate discriminatory techniques.

The technique of allozyme electrophoresis provided an objective method for the delineation of species boundaries where the species were found in sympatry. For allopatric populations an objective evaluation of the levels of genetic variation both within and between OTUs allowed a conservative estimation of species boundaries. The ease of interpretation of the results coupled with the presentation of phylogenetic implications of the results, render this technique most valuable to systematics. Practically, however, electrophoresis must be performed in conjunction with classical taxonomy, as this study has indicated. Where electrophoresis identified species boundaries, diagnostic morphological characters were found which enabled the species to be identified in the absence of electrophoresis.

The technique of multivariate morphometrics was found to be the least powerful method for discriminating groups, especially where clearly distinct electrophoretic OTUs could not be separated morphometrically. However the discriminatory power of the morphometric analysis might have been improved by the inclusion of diagnostic character states such as the presence or absence of sternal pores. These diagnostic characters were not included in the morphometric analysis so as to avoid circularity, since these characters were included in the initial selection of OTUs and the section on classical taxonomy. Consequently, excepting the possibility of correlations between morphometric and 'classical' characters, the two techniques of morphometrics and classical taxonomy are completely independent of one another.

In theory therefore, the three techniques were independent of one another. Rather than use each technique to individually delineate species boundaries and then compare the results of the each of them, in this thesis the three techniques were used sequentially to establish the species boundaries.

Section 3.1 INTRODUCTION

The overall aim of this chapter was to devise a hypothetical phylogenetic tree for the species in the genus *Engaeus* and its related genera by using information from two sources, namely the results of the electrophoretic analysis (presented in Chapter 2) and a cladistic analysis of morphological data.

The elucidation of such a hypothetical set of relationships has allowed an examination of the implications of the proposed phylogenetic relationships between the species of *Engaeus*, namely the modification with descent (or the evolution) of some important morphological characters, and the relationship between the ecological affinities of species and their proposed phylogenetic affinities. In addition the study has facilitated a discussion of the higher order classification of the Parastacidae.

Phylogeny of Freshwater Crayfish

In this thesis no attempt has been made to formally change the higher order classification as it was last portrayed by Hobbs (1974; see Chapter 1). Hobbs (1974) depicted the phylogeny of the Astacidea using synapomorphic characters such as the sexual structures and secondary sexual characteristics of the male and the structure of the sternum and gills. His interpretations are not considered to be in dispute.

Attempts to reconstruct the phylogeny of the genera of the Parastacidae have been limited in the past to only selected genera and the use of ecological and distributional data (see for instance the notions of Smith, 1912, and Smith and Schuster, 1913, cited in Chapter 1 of his thesis). The assertion of Riek (1959) that species of the genus *Engaeus* in particular, and the family Parastacidae in general, arose from a marine ancestor of the Axiidae has not been substantiated (see Bishop, 1967).

The work of Riek (1972) was certainly more thorough than the previous attempts. Riek based his work on morphological characters such as the nature of the cephalothoracic grooves, the penes, and the orientation of the chelae, and distributional data, and constructed a dendrogram which he interpreted as the phylogeny. His selection of characters resulted in the wide separation of *Geocharax* and *Gramastacus* from *Engaeus*, *Engaewa* and *Tenuibranchiurus*, with the former genera being allied to *Cherax*, *Paranephrops* and *Parastacoides*, whilst the latter genera were associated with *Parastacus*.

Patak and Baldwin (1984) used biochemical techniques, specifically an immunochemical comparison of the haemocyanins, to derive a phylogeny of some of the Australian Parastacidae. Their results showed that the haemocyanins of *Geocharax*, *Gramastacus* and *Engaeus* were more similar to each other than they were to that of any other parastacid, and that *Euastacus* and *Astacopsis* were more closely aligned to these genera than they were to *Cherax*, and thus disagreed with the findings of Riek (1972).

Ecology and Phylogeny

The fundamental element of cladistic analysis is the synapomorphic character state, or in other words, the derived character states which are held in common by two or more species (Hennig, 1966). One of the most difficult aspects of the cladistic approach is the choice of suitable characters. If for example, character states exist in a monophyletic lineage which have been derived independently as a historical response to similar environmental conditions, the selection of these as synapomorphic characters would result in a completely spurious phylogenetic interpretation. One way to avoid this problem is to use characters for which a definite polarity can be assigned; in other words, where primitive and derived states can easily be elucidated. In addition, one can use a technique which is completely independent of morphology, for instance electrophoresis, to create the initial clusters of species in a phylogenetic tree, and in doing so, validate the inclusion of morphological characters at the primary levels at least. This methodology has been followed here.

Having constructed a tree by deliberately removing the 'ecological' effects, we can test to see whether, in fact, ecological circumstances have contributed to the phylogenetic relationships between the species. The ecological classification of the burrows of freshwater crayfish proposed by Horwitz and Richardson (1986) provides ready-made ecological information which can be tested. For instance, are species that occupy type 3 burrows more closely related to each other than they are to other species?

Section 3.2 METHODS

A dendrogram was constructed by examining the electrophoretic results for groups of closely related species, by evaluating these groups using a cladistic analysis of morphological characters, and then by using the same cladistic analysis to establish the more distant relationships between the species groups and other genera of freshwater crayfish.

Electrophoretic Results

The electrophoretic results produced in Chapter 2 gave phylogenetic interpretations of the Tasmanian and Victorian OTUs; the OTUs were combined into groups where OTUs were obviously more closely related to each other than they were to other OTUs. Following the final delineation of species, most of these OTUs can now be named and put into species groups which again contain species which are obviously more closely related to each other than they are to other species. The species groups, their component species (with a species code) and the electrophoretic OTU groups are given below:

phyllocercus group:

OTUs **1-2**; *E. phyllocercus* (VPH)
OTU **3**; *Engaeus* VRJ (VRJ)

cunicularius group:

OTUs **4-5**; *E. fultoni* (VF)
OTUs **6-15**; *E. cunicularius* (VQ)
OTU **16**; *Engaeus* VQ9 (VQ9)
OTUs **17-20**; *E. quadrimanus* (VM)
OTUs **21-23**; *Engaeus* VS (VS)
OTUs **24-26**; *E. sericatus* (VSV)

hemicirratulus group:

OTUs **27-31**; *E. hemicirratulus* (VH)
OTUs **35-38**; *E. cymus* (VCY)

victoriensis group:

OTU **39**; *Engaeus* VAFA (VAFA)
OTUs **40-44**; *E. affinis* (VAFB)
OTUs **45-46**; *E. victoriensis* (VV)
OTUs **47-49**; *E. tuberculatus* (VT)
OTU **50**; *E. urostrictus* (VUO)

sternalis group:

OTU **52**; *E. orientalis* (VO)
OTU **53**; *E. sternalis* (VSN)
OTU **54**; *Engaeus* VSL (VSL)

fossor group:

OTUs **72-73**; *Engaeus* TBZ (TBZ)
OTU **74**; *Engaeus* TF (TF)
OTU **75**; *Engaeus* TB (TB)
OTU **76**; *Engaeus* TM (TM)
OTUs **78-79**; *E. fossor* (TG)

cisternarius group:

OTUs **80-81**; *E. cisternarius* (TH)
OTU **82**; *Engaeus* TN (TN)

leptorhynchus group

OTU **51**; *Engaeus* TA (TA)
OTU **77**; *Engaeus* TQ (TQ)
OTU **84**; *E. leptorhynchus* (TC)

In addition six species were not associated with any other species into groups:
E. laevis (VLA); OTUs **32-34**
E. strictifrons (VSF); OTUs **55-56**
E. australis (VAU); OTU **57**
E. lyelli (VLY); OTUs **58-62**
Engaeus TJ (TJ); OTU **71**
Engaeus TD (TD); OTU **83**

Morphological Data

The elucidation of uniquely derived shared character states (synapomorphies) in apparently monophyletic groups of species leads to a prediction of a hypothetical ancestor which exhibited the primitive character condition, plus a speciation event which produced lineages (or species); of these lineages one is said to have retained the primitive condition whilst the other has derived the unique state. Subsequent monophyletic groups, or clades, can be dealt with identically until a composite tree has been formulated which includes all the taxa. In the analysis presented here, the cladistic approach, using morphological characters, has been applied first to the electrophoretic groups in an attempt to produce a dendrogram which is compatible with the electrophoretic results, then an attempt was made to determine the relationships between these groups by using the same cladistic approach.

This section deals with the morphological characters which provide such information. The primitive and derived conditions of each character will be given, but the monophyletic groups and 'unique' status will not be elaborated upon here; the discussions will therefore centre on the theoretical polarity of the characters rather than their direct application in the cladistic analysis (which has been given in the RESULTS).

In addition, a discussion of the historical use of each character in parastacid phylogenetic reconstruction has been given where applicable.

The morphological features of freshwater crayfish, including their associated terminology and overall variability, are outlined in greater detail in Chapter 5.

GROOVES ON THE CARAPACE

The grooves or furrows on the carapace have been the focus of much attention from biologists who have examined both the extant and fossilized forms of the decapod crustaceans, and their developmental patterns have provided many authors with data to hypothesize the phylogenetic relationships amongst these organisms (see for example the works by Forster, 1966; Glaessner, 1960, 1969; Secretan, 1960a, 1960b, 1964, 1966). Whilst there has been no general consensus as to the outcome of these studies, they have all discussed a general tendency throughout decapod evolution towards the reduction and simplification of the grooves. In parastacid phylogeny probably the most important character is the relationship between the cervical groove with the postcervical and branchiocardiac grooves, as exemplified by the phylogeny of Riek (1972). In the present study, however, only two character states are recognized; the primitive character state is where the postcervical and branchiocardiac grooves are widely separated from the cervical groove, whilst the derived condition is for these grooves to become fused. Such an assertion is based on the assumption that once grooves have fused then they are unlikely to separate again. In addition, the shape of the cervical groove at the meson of the carapace has been used in the phylogeny proposed by Riek (1972). In this work I will assume that the primitive character state is a U-shaped groove, whilst the derived state is a V-shaped groove; this is based upon the assumption that an alteration in the degree of vaulting of the carapace (as a result of an increase in the size of the branchial chamber) will alter the shape of the groove from U- to V-shaped and that having done so the shape of the cervical groove will not revert to its former condition.

STERNAL PORES

Each lateral process at the first, second, third and fourth pereiopodal position on the sternum may or may not exhibit a pore. Such pores have been used as taxonomic characters (see for example Clark, 1936a; Suter, 1977a; this thesis) but they have not been included as characters in a phylogenetic evaluation of parastacids. Suter (1975) performed a histological examination of the large pores of *E. cisternarius* and whilst detecting the presence of mucopolysaccharides, was unable to draw conclusions as to their function; his tentative suggestion that their secretions allow the crayfish to water-proof the lining of their burrows can be considered as unlikely since other crayfish occupying type 3 burrows in very similar soils do not possess pores (for example *E. hemicirratulus*). For this study, on each lateral process the primitive condition is taken to be the presence of a pore whilst the derived condition is the loss of that pore; this is based upon the assumption that sternal pores have arisen only once in the evolution of the parastacids, and that once lost they have remained lost.

STERNAL KEEL

The presence of a keel running anteroposteriorly between the lateral processes is also assumed to have arisen only once in the evolution of the parastacids and having done so its subsequent loss can be considered as the derived condition whilst its presence can be taken

as being the primitive condition. One problem with the use of the keel as an important character is that its modification through descent, particularly within the genus *Engaeus*, has been gradual so that its reduction can only be interpreted as being shades of a presence rather than only an presence-absence dichotomy.

ANNULUS VENTRALIS

Prior to this study the annulus ventralis had not been described in parastacids; here its primitive condition has been interpreted as the undivided smooth rim which runs around the posterior of the lateral processes of the fourth pereiopods; its derived condition is an increased structural complexity where it becomes divided and conspicuously swollen and/or elongated.

2nd ABDOMINAL SOMITE - FLAP

Horwitz (1987, in press, Appendix I) described the nature and the occurrence of the subcalcified anteroventral extension of the pleura of somite 2 of the abdomen and assumed that this character has arisen only once in the evolution of the Parastacidae. The primitive condition for these species is therefore the absence of the flap, and the derived condition is its presence. One species of *Engaeus* appears to have secondarily lost this flap (*Engaeus* VRJ).

EXOPODITE OF THE THIRD MAXILLIPED

Three distinct character states are found for this appendage; the most primitive of which is the multiarticulate condition of the exopodite with both a flagellum and a shaft. The flagellum may be lost so that only a shaft is displayed, and the most derived condition is for the entire appendage to be lost. Therefore, a definite polarity can be assigned to the modification of this appendage through descent; it is based on the assumption that having lost a flagellum, in the first instance, and a shaft in the second instance, these structures of the appendage cannot be regained. This character has been used in taxonomic keys for the genus *Engaeus* (for example Clark, 1936a; Riek, 1969); in addition Riek (1969) implied diagrammatically that his "suggested relationships and species groups of *Engaeus*" were based on a presence or absence of this appendage.

GILLS

The primitive gill formula for the Parastacidae is assumed to be 21 + ep and all values below this level are therefore derived; this assertion is based on the assumption that once a gill has been significantly reduced in size or lost it cannot be regained.

TRANSVERSE SUTURE OF THE OUTER RAMUS OF THE UROPOD

The primitive condition for this character is its presence and its absence is a derived character; this polarity is based on the assumption that it cannot be regained after being lost.

ANTENNAL SCALE

Riek (1969) used the character of the antennal scale in his "suggested relationships and species groups of *Engaeus*", where he separated his so-called *fossor* group from the remaining species. In this study the primitive condition has been taken as a fully developed appendage which extends to at least the middle of the distal segment of the antennal

peduncle, and where it displays a terminal, conical spine. A reduction in size of the scale, particularly where it is associated with a loss of the spine, has been taken as a derived character state.

CHELAE

Tuberculation of the propodal palm of the chelae will be interpreted as a derived character state, where a primitive state is the absence of tubercles on the propodus. This is based on the assumption that having arisen, the tuberculation will never be completely lost again and a vestige of the tuberculation is bound to remain.

Section 3.3 RESULTS

Dendrogram

Figure 1 shows the dendrogram for the species of *Engaeus* and its related genera. At each node (represented by a circle and a number) is a hypothetical ancestor which held the primitive character state or states. Extending from each node are two or more monophyletic lineages (or clades); each lineage terminates in a square and a species code to represent a species. The length of each line or lineage is arbitrary; the lengths are not meant to imply a time since divergence.

The following is an account of each division in the dendrogram, where the primitive or plesiomorphic character state is given for each node, followed by the synapomorphic character state (with the components of the clade). Terminal bifurcations are not resolved with character state analysis since a two taxon tree is inherently already resolved (Wiley, 1981).

The tree commences at the proposed origins of the sternal pores, where the ancestor did not exhibit sternal pores, nor did the ancestral taxon (see below); the sternal pores are found in descendants of the ancestral taxon 3.

- branchiocardiac and cervical grooves remaining separated
  b - *Paranephrops.*
- branchiocardiac and cervical grooves fused
  a - *Astacoides, Astacopsis, Euastacoides, Euastacus, Parastacoides, Parastacus, Samastacus.*

3 - absence of abdominal flap on reproductively-active females
  c - *Cherax*
- presence of abdominal flap on reproductively-active females
  4

4 - Annulus ventralis undivided or only partially divided
  5
- Annulus ventralis fully developed and divided
  6

5 - Continuation of keel between lateral processes of 4th pereiopods as thin ridge; processes sloping posteriorly
  - d - *Geocharax*
  - e - *Gramastacus*

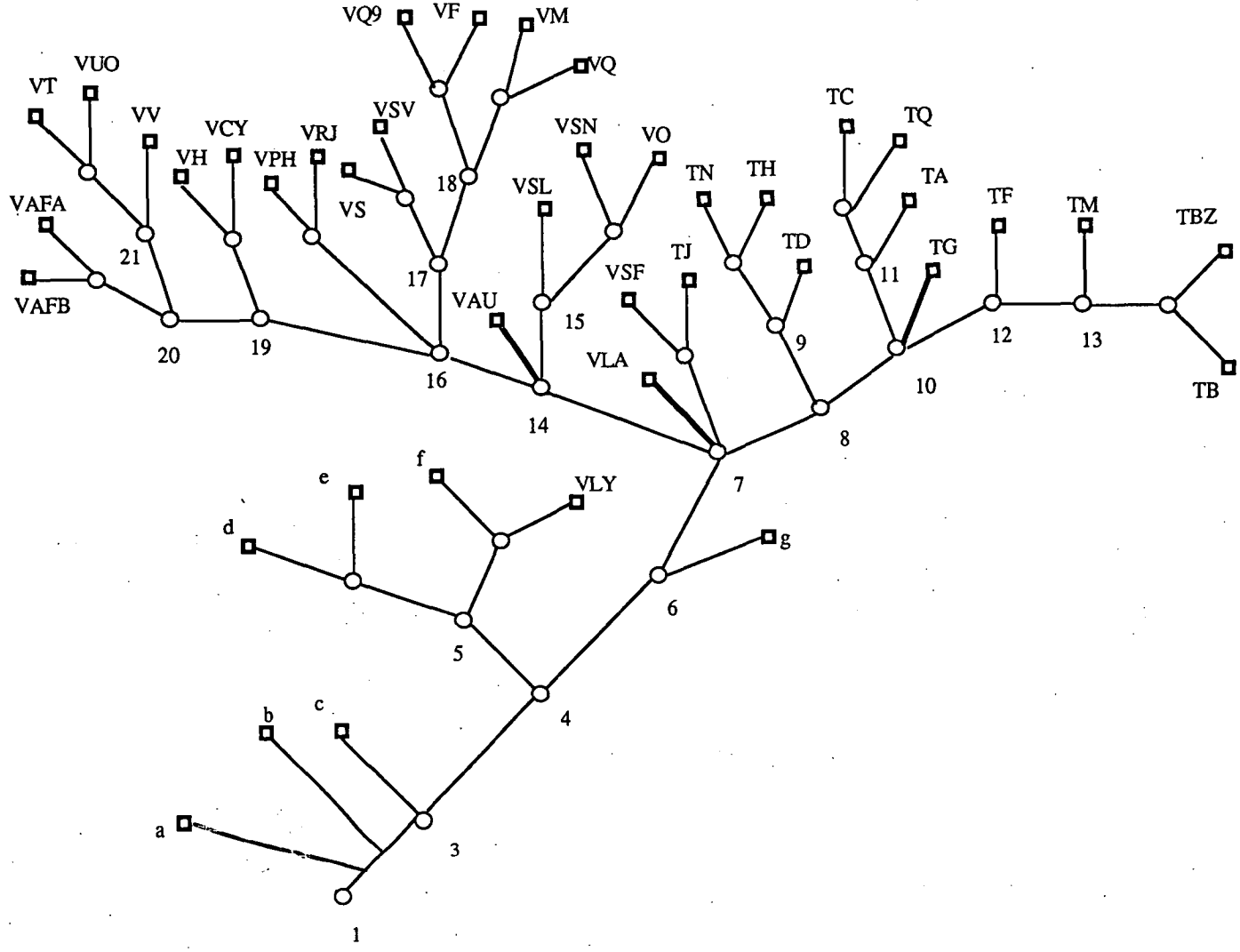


FIGURE 1: Dendrogram produced from electrophoretic results and cladistic analyses of morphological characters. See text for explanations. Species codes are given in the Methods (under the heading 'Electrophoretic Results').

5 (cont.)

- Continuation of keel between lateral processes of 4th pereiopods as broad ridge to posterior edge

  - f - *Tenuibranchiurus*

  - VLY - *Engaeus lyelli*

6 - Cervical groove remaining U-shaped at meson

  - g - *Engaewa*

- Cervical groove broadly V-shaped or V-shaped

  7

7 TRICHOTOMY (plus one unresolved species)

- Lateral processes of third pereiopods swollen with very large ovoid pores (when pores present); lateral processes of fourth pereiopods with elongate pores usually opening ventrally (when pores present); annulus ventralis not longer than broad

  8

- Pores on the lateral processes of the third and/or fourth pereiopods either small and ovoid, or lost; annulus ventralis not longer than broad

  14

-Annulus ventralis conspicuously enlarged, at least as long as broad

  - VSF - *E. strictifrons*

  - TJ - *Engaeus* TJ

UNRESOLVED

  - VLA - *Engaeus laevis*

8 - Pores on lateral processes of 4th pereiopods not everted (when present)

  10

- Pores on lateral processes of 4th pereiopods everted (when present)

  9

9 - Transverse suture of outer ramus of uropod present

  - TD - *Engaeus* TD

- Transverse suture of outer ramus lost

  - TN - *Engaeus* TN

  - TH - *E. cisternarius*

10 (including one unresolved species)

- Sternal keel remaining high and well developed

12

- Sternal keel reduced in height (to below the height of the articulations of the third pereiopods)

11

UNRESOLVED

- TG - *E. fossor*

11 - Retention of multiarticulate exopodite of third maxilliped; sternal pores present

- TA - *Engaeus* TA

- Exopodite of third maxilliped reduced to shaft; pores lost

- TQ - *Engaeus* TQ

- TC - *E. leptorhynchus*

12 - Lateral processes of first and second pereiopods with pores

- TF - *Engaeus* TF

- Lateral processes of 1st and 2nd pereiopods without pores

13

13 - Lateral processes of 4th pereiopods with pores

TB - *Engaeus* TB

TBY - *Engaeus* TBY

- Lateral processes of 4th pereiopods without pores

TM - *Engaeus* TM

14 (including one unresolved species)

- Lateral processes of the 4th pereiopods separated at posterior edge by central lobular extension (posterior remnants of keel)

15

- Keel entirely absent between lateral processes of 4th pereiopods

16

UNRESOLVED

- VAU - *E. australis*

15 - Exopodite of the third maxilliped retained as multiarticulate

VSL - *Engaeus* VSL

15 (cont.)

- Exopodite of the third maxilliped reduced to shaft

VSN - *E. sternalis*

VO - *E. orientalis*

16 TRICHOTOMY

- Sternal pores retained

17

- All sternal pores lost, antennal scale not reduced

19

- All sternal pores lost, antennal scale reduced in size

- VPH - *E. phyllocercus*

- VRJ - *Engaeus* VRJ

17 - Setae on propodal palm present on both lateral and mesal surfaces

18

- Setae on propodal palm present only on mesal surfaces (lost on lateral surface)

- VS - *Engaeus* VS

- VSV - *E. sericatus*

18 - Ventral surface of propodus without tubercles

- VQ - *E. cunicularius*

- VM - *E. quadrimanus*

- Propodal palm tuberculate along ventral surface

- VF - *E. fultoni*

- VQ9 - *Engaeus* VQ9

19 - Sternal keel remaining at least as high as articulation height of lateral processes of third pereiopod

20

- Sternal keel reduced to lower than articulation height of lateral processes of third pereiopod or lost completely

- VH - *E. hemicirratulus*

- VCY - *E. cymus*

20 - Posterior pleurobranch remaining large and fully developed

- VAFB - *E. affinis*

- VAFA - *Engaeus* VAFA

- Posterior pleurobranch reduced in size or rudimentary

21

21 - Propodal palm of chelae without setose tubercles

- VV - *E. victoriensis*

- Setose tubercles on propodal palm of chelae

- VT - *E. tuberculatus*

- VUO - *E. urostrictus*

Structure of Dendrogram

TRICHOTOMIES, UNRESOLVED SPECIES AND SYNAPOMORPHIES*

The dendrogram depicting phylogenetic relationships displays two trichotomies at nodes 7 and 16 and three unresolved species at nodes 7, 10 and 14.

The trichotomy at node 7 arises from the recognition of a synapomorphic character for *E. strictifrons* and *Engaeus* TJ (the structure of the annulus ventralis), and a failure to conclusively include these two species in either of the two other lineages. The position of these two species in the dendrogram correlates well with their isolation in the Victorian Study and Tasmanian Study, respectively, in the electrophoretic analysis.

At node 16 the lineage containing the *phyllocercus* group could not be conclusively allied with either of the two other lineages. Its position in this trichotomy also arises from the central position which it assumes in the Wagner tree relative to the two other lineages (Figure 5B, Section 2.2).

E. laevis could not be grouped unequivocally with any other species electrophoretically, despite showing not distant relationships to both *Engaeus* TA in the Wagner trees in the Victorian Study, and *E. hemicirratulus* in the UPGMA for the Victorian Study. Morphologically it appears to be most closely allied to *Gramastacus* with a similar penes size and structure of the lateral processes at the 4th pereiopods. Its central position in the dendrogram reflects the enigmatic nature of this species.

E. fossor is most closely allied to *Engaeus* TF in the electrophoretic analysis but no synapomorphic feature could be found to link it with this species. Its position at the node 10 accounts for a sharing of the derived character plus an electrophoretic affiliation to the 'primitive' lineage.

E. australis is incorporated distantly in the electrophoretic results for the Victorian Study, but with the other Victorian species in the Tasmanian Study. Its morphological features include a combination of 'primitive' (for instance a multiarticulate exopodite of the third maxilliped) and 'derived' (such as a lack of sternal pores and a very low sternal keel).

On two occasions, characters which were recognised as synapomorphies are interpreted as having undergone a secondary modification of structure since divergence of the hypothesized ancestor (and these characters therefore need not be recognised as synapomorphic

* At each node which represented more than three taxa, synapomorphies were given for one of the two (or three) branches, rather than for each branch as advocated by Wiley (1981). For the purpose of this thesis, the remaining branches were said to be intuitively substantiated; future attempts will determine synapomorphies for each branch.

in the strict sense of the word). The most important of these secondary modifications is found for the Tasmanian species *E. leptorhynchus*, *Engaeus* TQ and *Engaeus* TM which appear to have lost their sternal pores; their position in the dendrogram is based on the assumption that the electrophoretic groupings to species with the correct synapomorphic character state (*Engaeus* TA for the former two species, and *Engaeus* TB and *Engaeus* TBZ for the latter species) are viable and represent true genealogies.

The other example occurs at the node where *E. phyllocercus* and *Engaeus* VRJ share the 'unique' character of the reduction in size of the antennal scale. However, *E. urostrictus* in another of the lineages, also exhibits this character state. The use of this character is therefore based on the assumption that the character state shown by *E. urostrictus* arose independently from that state which arose in a common ancestor of *E. phyllocercus* and *Engaeus* VRJ; the electrophoretic evidence supports this assumption.

WITHIN SPECIES GROUPS

The cladistic approach has confirmed the relationships and cohesiveness within the species groups as defined by the electrophoretic results. The exceptions to this are outlined above.

BETWEEN SPECIES GROUPS

The between species group relationships are also compatible with the implications of the electrophoretic work, with for instance a common ancestor linking the *cunicularius*, *affinis*, *hemicirratulus* and *phyllocercus* groups. Similarly a common ancestor links the *leptorhynchus* and *fossor* groups. *Engaeus* TJ and *E. strictifrons* are linked together to form a previously unidentified group.

The *cisternarius* group and *Engaeus* TD can be associated with other Tasmanian species on the basis of the pore shapes and they have been placed at the base of the 'Tasmanian branch' because of their distant incorporation in the electrophoretic studies.

HIGHER ORDER RELATIONSHIPS

The higher order relationships show that *Geocharax* and *Gramastacus* are closely linked, as are *Tenuibranchiurus* and *E. lyelli*, and in both cases the structure of the annulus ventralis and the lateral processes of the 4th pereiopods are important in their positions.

Section 3.4 DISCUSSION

Terminology

There is continuing debate over the terminology for dendrograms, or branching diagrams. The structure of Figure 1 conforms to the basic principles of cladistics as outlined by Hennig (1966), since individual speciation events and common ancestry have been identified by synapomorphies using morphological characters. Another interpretation of Figure 1 might be a branching diagram which is a summary of morphological pattern where there is no time scale and the nodes imply synapomorphies only, not common ancestry, as discussed by Patterson (1982); under these circumstances the term 'cladogram' might seem applicable.

However, the corroboration between the cladistic analysis and the electrophoretic results imparts an a priori connotation of the evolutionary relationships between at least the closely related groups of *Engaeus* species. For this reason (following the rationale used by Wiley, 1981) Figure 1 could be termed a 'phylogram' or a 'phylogenetic tree'.

In attempt to avoid the confusion over tree terminology, but retain some modicum of clarity, Figure 1 has been called a 'dendrogram depicting phylogenetic relationships'.

The dendrogram actually represents a large number of genealogical hypotheses, all of which are based on the assumptions of the polarity of morphological characters and the validity of the electrophoretic results, as outlined earlier. This simply reflects the conjectural nature of phylogenetic analyses in the absence of more exacting techniques (a fact which can be exempified here by the presence of trichotomies and unresolved species). The discussions and conclusions presented below must be examined in this light.

All of the zoogeographical aspects of the proposed phylogenetic relationships will be discussed in Chapter 4.

Character Modification

An important interpretation of the dendrogram is that many of the morphological features show a similar trend; those which exhibit a distinct polarity, for instance ones where ancestral and derived forms can clearly be elucidated, have undergone identical evolutionary modification in two or more lineages. The most obvious of these are the sternal pores, which have been completely lost on at least eight occasions, the most recent of which (judging by the degree of electrophoretic divergence) appears to be the split between *E. cisternarius* and *Engaeus* TN. There is additional evidence to suggest that the process of pore loss is an on-going one, with the species *E. quadrimanus* in East Gippsland exhibiting high proportions of individuals in a population with a reduced number of pores (see SPECIES DESCRIPTIONS, Chapter 6).

Similarly, the exopodite of the third maxilliped has been reduced at least to a shaft on seven separate occasions in the evolution of the genus *Engaeus*; again we can identify

a species where the process of the modification is an on-going one, for the species *E. fultoni* which has almost lost the occurrence of the flagellum in any one population.

Other examples of the modification of morphological characters in more than one lineage include

i) the transverse suture of the outer ramus of the uropod (which has been lost independently on three occasions, for *E. hemicirratulus*, *E. cisternarius* and *Engaeus* TN, and *Engaeus* TA),

ii) the reduction in size of the antennal scale (for *E. urostrictus*, *E. phyllocercus* and *Engaeus* VRJ and finally *E. cisternarius* and *Engaeus* TN),

iii) the reduction is size or loss of the posterior pleurobranch (which is reduced for *E. phyllocercus*, and *E. victoriensis*, *E. urostrictus* and *E. tuberculatus*, and has been lost for *E. hemicirratulus*), and

iv) the inner flagellum of the antennules which is lost for *E. hemicirratulus* and is in the process of being lost in the species *E. tuberculatus*.

Riek (1969) only produced a diagrammatical scheme to depict the phylogeny of species within the genus *Engaeus*; this diagram shows that (the ancestors of) the species with the primitive character of the multiarticulate exopodite of the third maxilliped, for instance *E. quadrimanus*, gave rise to two stocks, one with the synapomorphic character of the reduced antennal scale, and one with a reduced exopodite of the third maxilliped; this latter stock apparently gave rise to species where the exopodite has been completely lost. Kane (1964) presented a similar scheme. These authors therefore, according to the hypotheses in this thesis, have misinterpreted the morphological variation within the genus *Engaeus* to produce species groupings and 'suggested relationships' which have treated these characters as synapomorphies for the entire genus, rather than for separate lineages within the genus.

The mechanisms which have been involved to produce such parallelism in the modification of morphological characters is not known (but see below); perhaps the ancestors to the lineages have had a predisposition towards the loss or reduction of each of these morphological characters.

Higher Order Classification

The phylogram depicts the presence or absence of sternal pores as being the initial ancestral speciation event; in so doing it means that the cephalothoracic grooves have fused in two independent lineages, at least once at node 2 and once for *Cherax*. If this is assumed, then the parastacids can be divided into two major groups, one with sternal pores (but if without, then always exhibiting the flap on the abdomen), and one without sternal pores.

The first of these major groups are depicted in the dendrogram, showing that the genera *Engaeus*, *Engaewa*, *Geocharax*, *Gramastacus* and *Tenuibranchiurus* can all be linked by a common ancestor that evolved a secondary sexual characteristic of the female, namely the

subcalcified, anteroventral extension of the pleura of somite 2 of the abdomen. Reproductively-active females representing all of the Australasian parastacid genera have been examined and only species in the above five show this trait. It is proposed here that this fact alone is sufficient to group these five genera into a subfamily of their own. On the assumptions that this character has arisen only once and that the other genera have not had the character and subsequently lost it in the evolution of the parastacids, this classification has a phylogenetic basis to its existence (Horwitz, 1987, in press, Appendix I). This conclusion also corroborates those of Patak and Baldwin (1984) for the genera of *Engaeus*, *Geocharax* and *Gramastacus*. With *Cherax*, this major group could well be considered as a family of its own, with two distinct subfamilies, one containing *Cherax* and one containing the 5 closely allied genera.

E. lyelli is the only species currently recognised as being within the genus *Engaeus* which falls outside the main *Engaeus* stock (at node 7 of the dendrogram).

The structure of the sternum of *E. lyelli* is almost unique to the parastacid fauna, with the retention of the primitive characteristics such as the presence of pores (albeit only at the 4th pereiopods), the annulus ventralis which is rarely divided or only partially divided, and the nature of the keel between the lateral processes of the 4th pereiopods; it shares the latter two characters states with *Tenuibranchiurus* and this may be enough information to warrant the combination of these species into one genus.

Further information is required to determine the exact relationship between *E. laevis* and other species in the genus *Engaeus*. An undescribed species found near Wyong, New South Wales, by Chris Austin (Zoology Department, University of Western Australia) appears to belong to the genus *Gramastacus*, and since it also shows morphological similarity to *E. laevis*, it should therefore be included in any further analysis.

Ecology and Phylogeny

Figure 2 shows the dendrogram with the ecological classifications of Horwitz and Richardson (1986) superimposed on it. It shows that species capable of occupying type 3 burrows are present in both the Tasmanian and Victorian stocks of *Engaeus*, where they can be found in five lineages. Similarly, species capable of occupying type 1a or 1b burrows can be found in three separate lineages. This information shows that species from different lineages have adapted to the same burrow habitats in parallel. If one accepts that ecological parameters can affect the modification of crayfish morphology, then such ecological convergence may well have resulted in the morphological parallelism which was described above.

Possible phylogenetic misinterpretations of this convergence are exemplified by Suter (1977a) who suggested an affinity between *E. cisternarius* and *E. hemicirratulus* (which are both capable of occupying type 3 burrows) apparently on the basis of morphological characteristics.

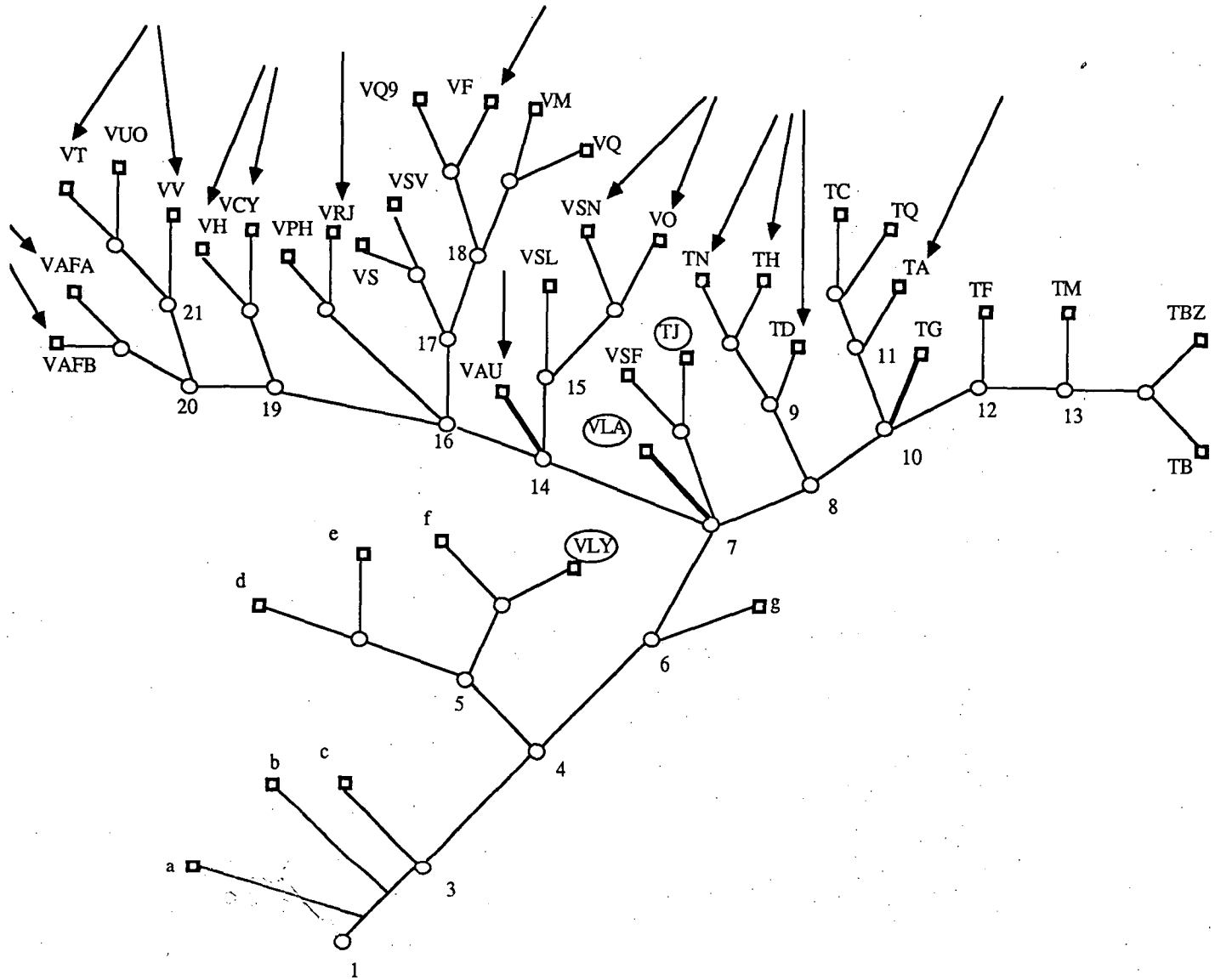


FIGURE 2: Dendrogram as depicted for Figure 1 except with species highlighted if they are capable of occupying type 3 burrows (arrowed), and if they are capable of occupying type 1a or 1b burrows (circled). See text for explanations.

Conclusions

A dendrogram depicting phylogenetic relationships was compiled from electrophoretic results and a supplementary cladistic analysis of morphological characters. A new higher order classification for at least some of the genera of the Parastacidae was suggested on the basis of the dendrogram and its corroboration with the immunological data of Patak and Baldwin (1984). Examination of clearly polarized morphological characters in relation to the dendrogram showed that evolutionary modifications of several features have occurred in parallel for species in the genus *Engaeus*. Similarly, ecological convergence was shown to occur for species in different lineages. No attempt was made to impart a time scale to the dendrogram.

Finally, each monophyletic lineage in the dendrogram represents a genealogical hypothesis. Each of these lineages can be tested or falsified by critically examining the synapomorphic character(s) (or the assumptions governing their use) at each node. Alternatively, the entire tree can be tested by applying more stringent phylogenetic techniques.

CHAPTER 4 - ZOOGEOGRAPHY

Section 4.1 INTRODUCTION

As described in Chapter 1, the origin of the parastacid fauna, along with other Crustacean groups, may have been in Gondwanaland, thus accounting for their prevalent distributions in the southern hemisphere. It is beyond the realm of this study to explore this relationship further, merely to add that the five genera of *Engaeus*, *Engaewa*, *Tenuibranchiurus*, *Geocharax* and *Gramastacus* are hypothesized to be more closely related to each other than they are to any other genera (see Chapter 3), and that this 'natural' group occurs exclusively in Australia and Tasmania. The primary objective of this Chapter is to present the distributions of these five genera. More specifically, the objective is to examine the zoogeography of the species in the most diverse genus, *Engaeus*; the zoogeography of the other four genera are included where possible and/or relevant.

Zoogeography is the study of the spatial and temporal factors which have influenced the distribution of animals (Wiley, 1981). The most difficult task of the phylogenetic zoogeographer is to unravel these factors. Having elucidated their taxa, most authors pursue their task in two stages. The first is an ecological approach, and this includes the dispersal capabilities of each species and the environmental parameters which appear to correlate with the species' distribution. The second is a historical approach, which takes the above correlations and attempts to apply them to historical, often vicariant, events so that an explanation of present-day distributions can be provided. These general approaches have been followed in this chapter. Factors which are most likely to have had an affect on the distributions of crayfish are discussed sequentially, under the following major headings:

Distributions of Crayfish Species

The actual distributions of each species in the genus of *Engaeus* are plotted on maps and a brief description of each distribution is given, along with a discussion of the distributions of the other 4 genera. This information provides the base-line data for the remaining discussions.

Rates of Dispersal

The rate of dispersal of individuals in a species is important since it enables an observer to estimate the species' capacity to colonize an adjacent area, given the initial information that it occupies a certain habitat and a known region. Information from this 'capacity to disperse' can be used to answer such questions as "why is species A in region X and not region Y when the habitat is apparently identical?".

The study of dispersal is, however, one in its own right. To obtain actual data on the distance covered by an individual in a certain period of time and then extrapolate this information to the entire species is a complex process, and such data has certainly not been obtained in this study. Information on dispersal rates was collected from indirect sources and has been presented in general terms only.

Interactions Between Species

Distributions are of primary importance from a systematic point of view, since they may provide additional evidence with which the systematist can infer reproductive isolation between two phenotypic populations. Beyond this, at the species level, answers to such questions as "do the species overlap in their ranges?" or "do they coexist?" can yield some interesting and important information, particularly on factors such as habitat utilization and modes of speciation. For instance in some cases, coexistence of two species may indicate a recent dispersal where at least one of the species has increased its distributional range (after Rosen, 1978). Comparative information from distributions can suggest possible physiological features of a species which limit that species to a certain geographical area or habitat, or they can suggest possible interactions between species. Such interactions are likely to be in the form of competitive exclusion or character displacement, although experimentation is required to test any of these assertions. Thus the distribution or occurrence of one species might have an influence on that of another species, and the crayfish distributions presented in this chapter have been examined for any such apparent influences.

Regional Endemicity

One of the major questions in biogeography is 'is endemism geographically non-random?' (Endler, 1982). A positive answer to this question precedes an examination of the ecological factors (including both physical and biological components of the environment) which may have contributed to such non-random patterns. In doing so the ultimate aim of the investigation would be to find one or more of these factors which can predict an individual's occurrence in any given area. However, any correlations between a species' distribution and the occurrence of one of these factors needs to be tested further by experimentation to determine whether a species is actively selecting a habitat for the presence of that factor; such experimentations are beyond the scope of this work. Nevertheless, the distributions of species of the genus *Engaeus* are investigated from a regional aspect, to test for regional endemicity and preliminary discussions are given on the relationship between environmental parameters and the distributions of species.

Climatic History

In an attempt to answer the question "how did such distributions arise?" we need to have some information on the history of the region, in order to explain how populations of species became isolated from each other and later developed into separate species. A major key to the understanding of the region is the climatic history, since climates are, in some authors' opinions, the most fundamental environmental variable to have a bearing on animal distributions. Climates have a direct influence on sea-levels, on the production of soils and the type of vegetation community present in any particular area. As such, climates have a direct influence on the possible events which provide barriers between populations and species (vicariant events). The climatic history of an area is usually inferred from a combination of a number of sources, including geomorphology, soil science, oxygen isotope studies of fossil-bearing ocean sediments and pollen analysis of sediments (particularly the latter two methods). Such inferences are often plagued with theoretical difficulties; for instance

fossilized plant records are biased to wetter periods since they are better preserved in the anaerobic conditions of lake bottoms (Truswell and Harris, 1982). In addition the use of information to infer past distributions of organisms by the use of climatic factors which themselves have been hypothesized from sources such as fossils and pollen counts (= distributions of organisms) involves circular reasoning and should be avoided where possible (Galloway and Kemp, 1981). Nevertheless these studies are showing some consistent climatic trends and some such trends are discussed.

Particular attention is given to the effects of the climatic conditions in the Bass Strait region of Australia since it is here that the origins of the parastacids have been proposed (Riek, 1972). In addition, most biogeographers suggest that the Bass Strait, whether dry or not, was effective as a barrier between the migrations of fauna from Tasmania to Victoria or vice versa. For instance Goede et al., 1978 presented fossil evidence from the so-called 'Pleistocene megafauna' to conclude that Tasmania, prior to its isolation by the rising seas after the most recent glacial period, was already a refuge for relict populations. Crayfish distributions are examined in this light.

Synthesis and Discussion

All of the information above is basically descriptive in nature. The next step in this zoogeography was an interpretive phase where the information was assimilated to attempt to reconstruct the pattern of events which led to the present-day distributions of crayfish species. This necessitates the hypothesizing of general modes of speciation. These hypotheses are presented and later compared to existing ones both in theoretical terms and with respect to the Bass Strait region. A brief discussion is given on the potential for further research to test these hypotheses.

Finally, Cracraft (1982) stated that

> '...one cannot reconstruct the history of speciation of any designated group if the analysis is restricted to just that group; one must have at least some notion of general vicariance patterns for the geographical areas in question...'.

Consequently other groups of fauna are briefly examined to attempt to find congruent distributional patterns and regional endemicities. As a practical by-product of such an approach, areas of high endemicity for more than one group of organisms could be highlighted for conservational purposes.

Section 4.2 CRAYFISH DISTRIBUTIONS

A distribution map for each of the species in the genus *Engaeus* was compiled from records of precise localities, where such localities could be determined either from personal records or museum records, and where the collecting locality could be plotted on a 1:100 000 topographical map. Collecting localities which failed to meet this criterion, for instance those which simply stated a geographical region, were not included in the map; the bulk of the rejected information came from museum records.

The distribution maps show only a species' presence. Such maps show a gap where individuals of a species either DO NOT OCCUR (where they have been searched for but not found), or where they MAY OR MAY NOT OCCUR (but have not been searched for). This merely serves to exemplify the truism that there is no such thing as a completed distribution map. In this thesis it will be assumed that the gaps in each map represent an actual absence; this will be backed up with information in the text where relevant.

In this section the distribution of each species is represented by a map and discussed in the text where important features need to be highlighted. To facilitate the interpretive phase, the species are presented in their species groups as portrayed in Chapter 3. Some of the place names and geographical localities which are used in this section appear in Figure 1.

SPECIES DISTRIBUTIONS

The *phyllocercus* group:

E. phyllocercus (Figure 2) has a limited distribution, apparently being restricted to the north-western half of the Western Strzelecki Ranges in South Gippsland, Victoria.

Engaeus VRJ (Figure 2) also has a restricted range, being found only at the headwaters of creeks on the Eastern Strzelecki Ranges in South Gippsland, Victoria.

The *affinis* group:

E. urostrictus (Figure 2) appears to be restricted to the Dandenong Ranges, Victoria, although a specimen has been recorded from Mt. Donna Buang further to the east; its eastern extension of its range, therefore, may be underestimated.

E. tuberculatus (Figure 3) occurs in central-southern Victoria, extending in a longitudinal belt from the Dandenong Ranges in the west, to south of Mt. Baw Baw in the east.

E. victoriensis (Figure 3) is located in the foothills of the Dandenong Ranges and on Mornington Peninsula; the disjunct distribution is likely to be a result of insufficient sampling between the two areas.

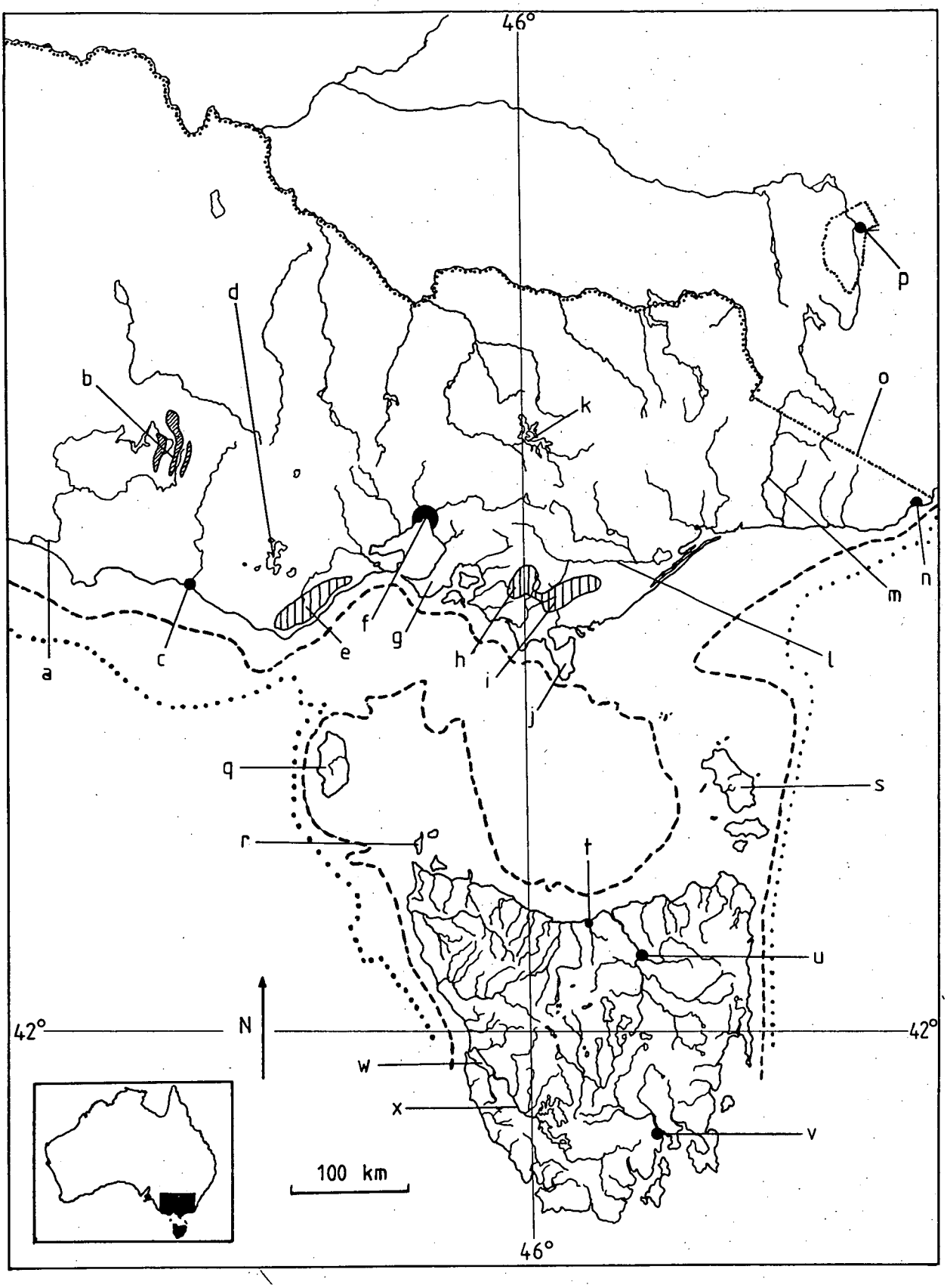
E. affinis (Figure 4) is widespread in the central-southern region of Victoria, where it can be found in the region of the upper reaches of the Yarra, Goulburn and La Trobe drainages.

Engaeus VAFA (Figure 4) appears as a distributional subset of the distribution found for *E. affinis*.

The *hemicirratulus* group:

E. cymus (Figure 5) has a broad geographical range in eastern Victoria where it can be found on both sides of the Great Dividing Range; the southern portion of its range occurs in

Figure 1: Map of Victoria, Tasmania and the Bass Strait showing some important geographical localities and features, and the depth contours of the Bass Strait region (where a dashed line represents a depth of 70 m and a dotted line represents a depth of 100 m; after Williams, 1974a);
a = the Glenelg River in Western Victoria,
b = the Grampian Ranges (presented as the approximate area over 500 m in elevation),
c = the town of Warrnambool on the Hopkins-Mt. Emu River system,
d = Lake Corangamite in the saline lake region of Western Victoria,
e = the Otway Ranges (presented as the approximate area over 200 m in elevation),
f = the city of Melbourne on Port Phillip Bay and at the mouth of the Yarra River,
g = Mornington Peninsula,
h and i = Strzelecki Ranges (presented as the approximate area over 200 m in elevation) and hereafter refered to as Western Strzelecki Range and the Eastern Strzelecki Range respectively,
j = Wilsons Promontory,
k = Lake Eildon on the Goulburn River,
l = La Trobe River in Gippsland, flowing into Lake Wellington,
m = lower reaches of Snowy River in East Gippsland,
n = Mallacoota Inlet,
o = Victoria-New South Wales border,
p = the city of Canberra in the Australian Capital Territory,
q = King Island along the western portion of Bass Strait,
r = Hunter Island, part of the Hunter Group which comprises three islands in all,
s = Flinders Island in the Furneaux Group of islands along the eastern side of Bass Strait,
t = town of Port Sorell in central-northern Tasmania,
u = town of Launceston on the Tamar River,
v = city of Hobart in southern Tasmania,
w = Macquarie Harbour on the west coast of Tasmania,
x = the lower reaches of the Gordon River.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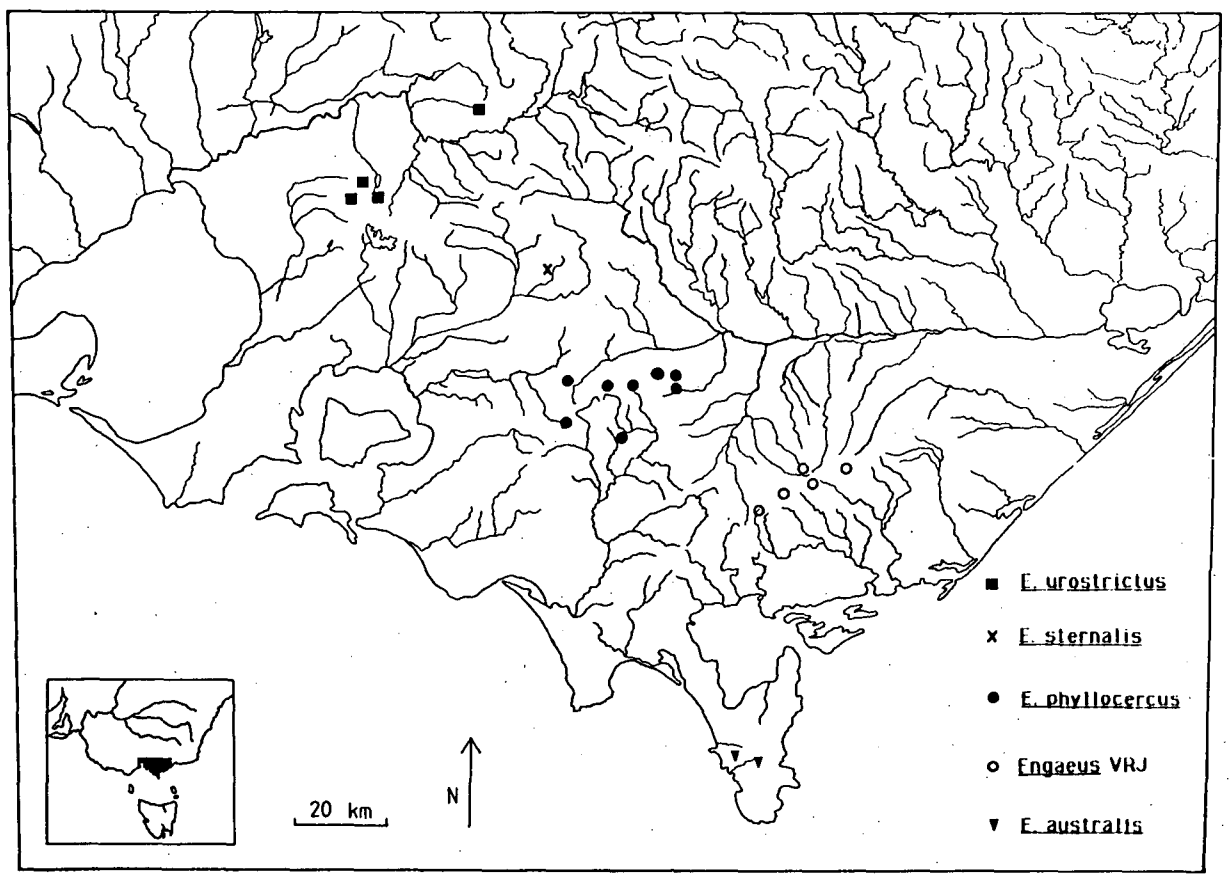


Figure 2: Distributions of 5 species of *Engaeus* in southern Victoria (*phyllocercus*, *urostrictus*, *sternalis*, *australis* and *Engaeus* VRJ).

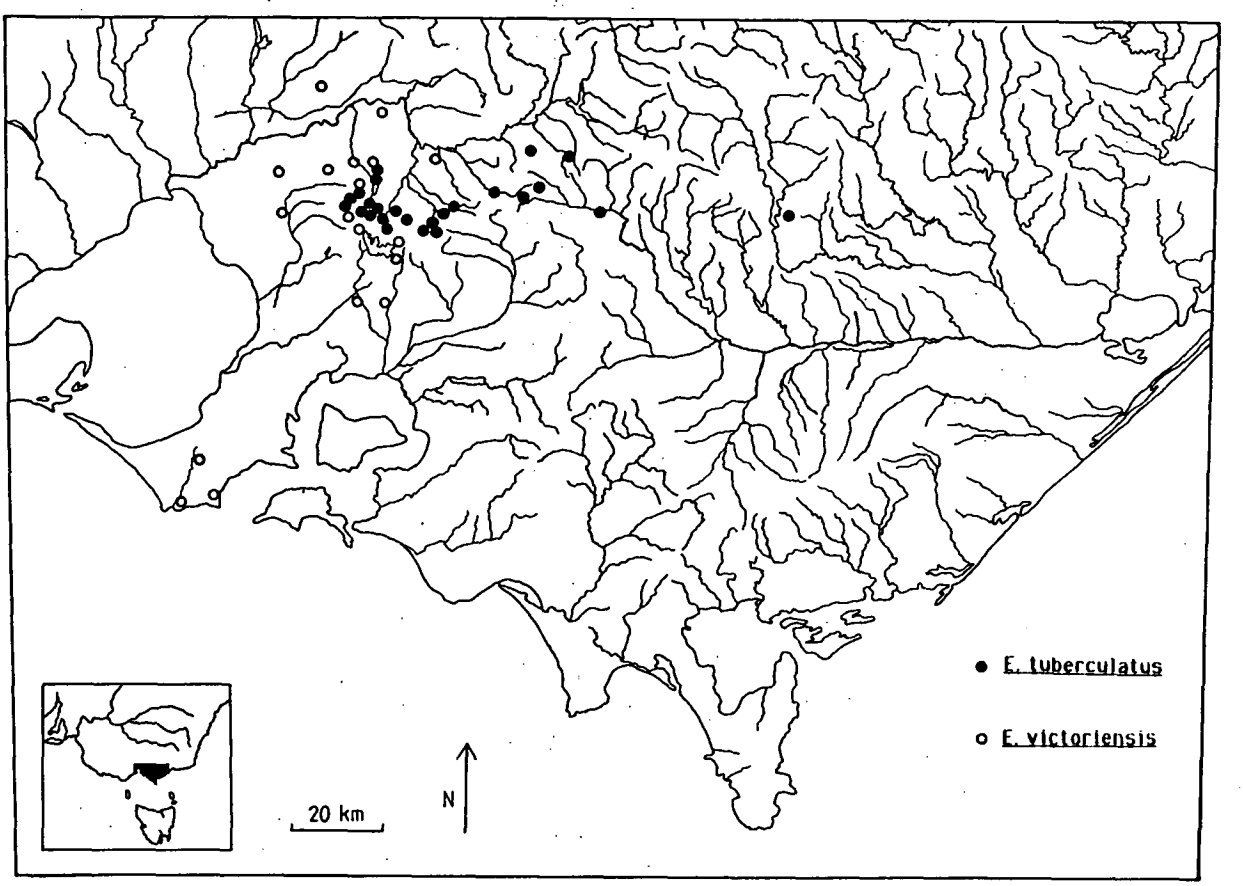


Figure 3: Distributions of 2 species of *Engaeus* in central-southern Victoria (*tuberculatus* and *victoriensis*).

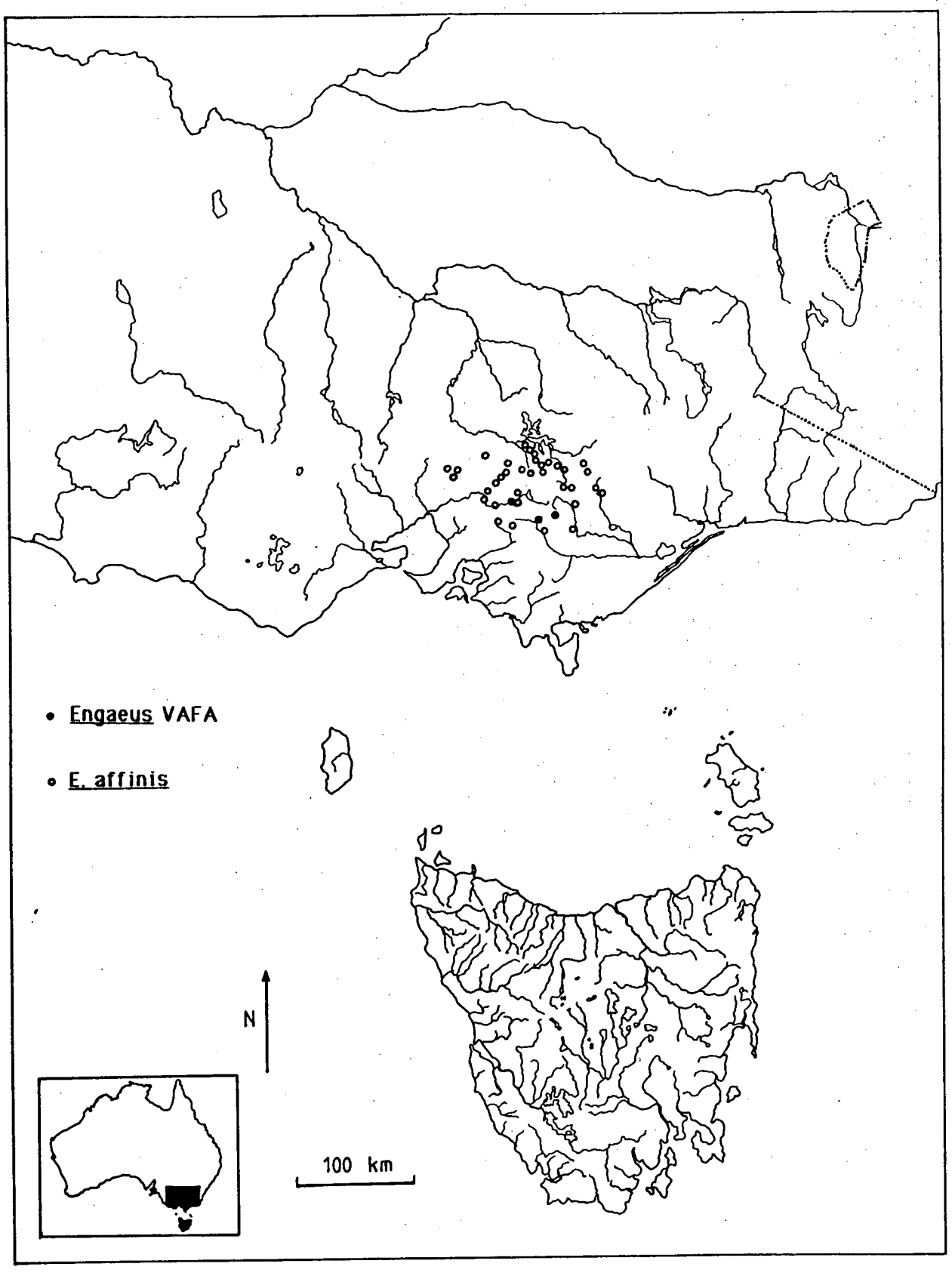


Figure 4: Distributions of 2 species of *Engaeus* in central-southern Victoria (*affinis* and *Engaeus* VAFA).

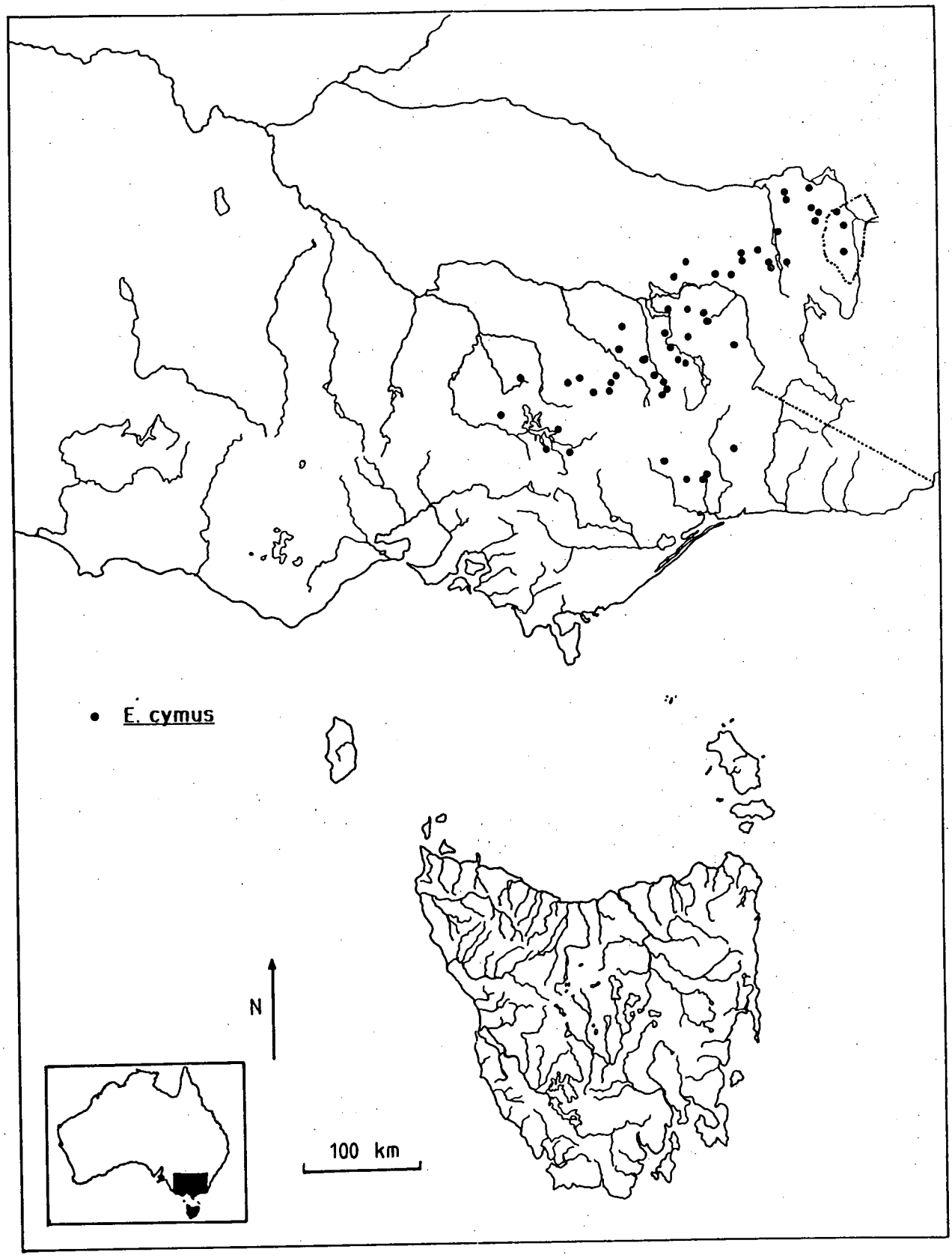


Figure 5: Distribution of *Engaeus cymus* in north-eastern Victoria.

the region north of Lakes Entrance and it appears to be disjunct from the remaining localities. However this gap may be due to the inaccesibility of this region of the Australian Alps where sampling was limited. Otherwise the species extends from the Black Range near Lake Eildon in Victoria, to the Burrinjuck Dam north of the Australian Capital Territory in New South Wales. Its north-western distribution is bounded by the Hume Highway.

E. hemicirratulus (Figure 6) occurs in both the central-southern and South Gippsland regions of Victoria, north and south of the La Trobe River. In South Gippsland it is found in both the Western and Eastern Strzelecki Ranges; a report of this species from Wilsons Promontory has not been confirmed.

The *cunicularius* group:

E. sericatus (Figure 7) is recorded from western Victoria, predominantly around the northern and western foot-hills of the Otway Ranges. It was not located in the creeks of the lake region (around Lake Corangamite) but was patchily located immediately north. A disjunct set of localities occurs around Port Fairy further to the west. The occurrence of type specimens from Croydon and Warburton, east of Melbourne is confusing and could not be substantiated (see Species Description of *E. sericatus*).

Engaeus VS (Figure 7) occurs near Geelong and around Ballarat in western Victoria. An outlying locality near Warrnambool extends its range further westwards.

E. fultoni (Figure 8) is found only in the Otway Ranges in western Victoria.

Engaeus VQ9 (Figure 9) occurs in the South Gippsland region of Victoria. Its range includes the southern portion of the Western Strzelecki Ranges and Wilsons Promontory.

E. quadrimanus (Figure 10) occurs over a wide area in eastern Victoria, south of the Great Dividing Range from near Mallacoota in the east to north of Melbourne; this range includes Wilsons Promontory and the northern and eastern foothills of the Strzelecki Ranges.

E. cunicularius (Figure 11) has a circum Bass Strait distribution, occurring on Flinders Island (and almost undoubtedly on other islands in the Furneaux Group), on King Island and on islands in the Hunter Group. In Tasmania it is found in the north-east and the north-west; these two areas are linked by a single locality near Port Sorell in the central-north. In Victoria it has been recorded on Wilsons Promontory, at patchily distributed localities at the coastal foothills of the Otway Ranges and abundantly between Melbourne, the Dandenong Ranges and the Strzelecki Ranges.

The *sternalis* group (including *E. australis*):

E. sternalis (Figure 2) is known from only one locality east-south-east of Melbourne, Victoria. Its cryptic habitat may be the reason for its apparently limited distribution.

E. australis (Figure 2) appears to be restricted to Wilsons Promontory.

Engaeus VSL (Figure 12) is also known from a very restricted range. Two localities are recorded, both of them from the western side of the Mallacoota Inlet in East Gippsland, Victoria.

E. orientalis (Figure 12) occurs in predominantly east of the Snowy River in East Gippsland, Victoria and makes short incursions over the border into New South Wales.

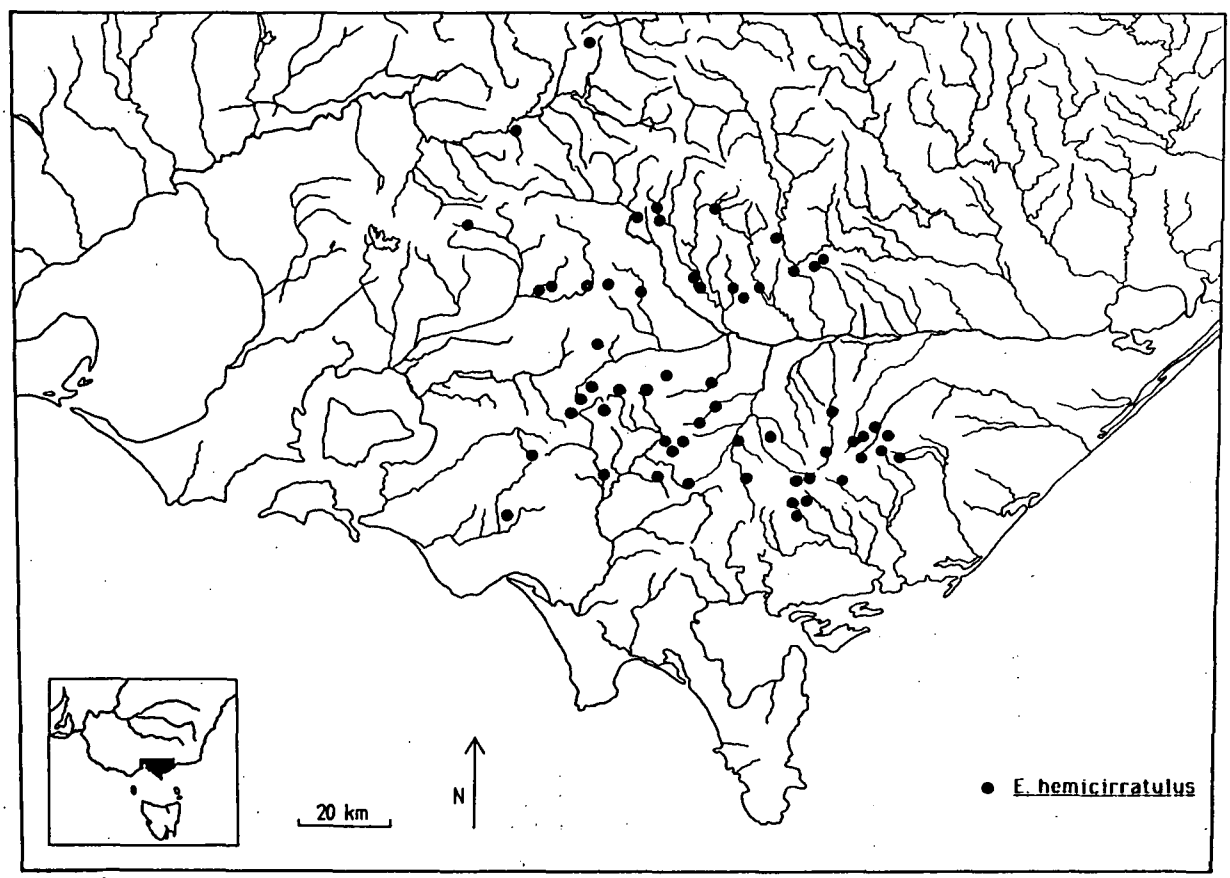


Figure 6: Distribution of *Engaeus hemicirratulus* in central- southern Victoria.

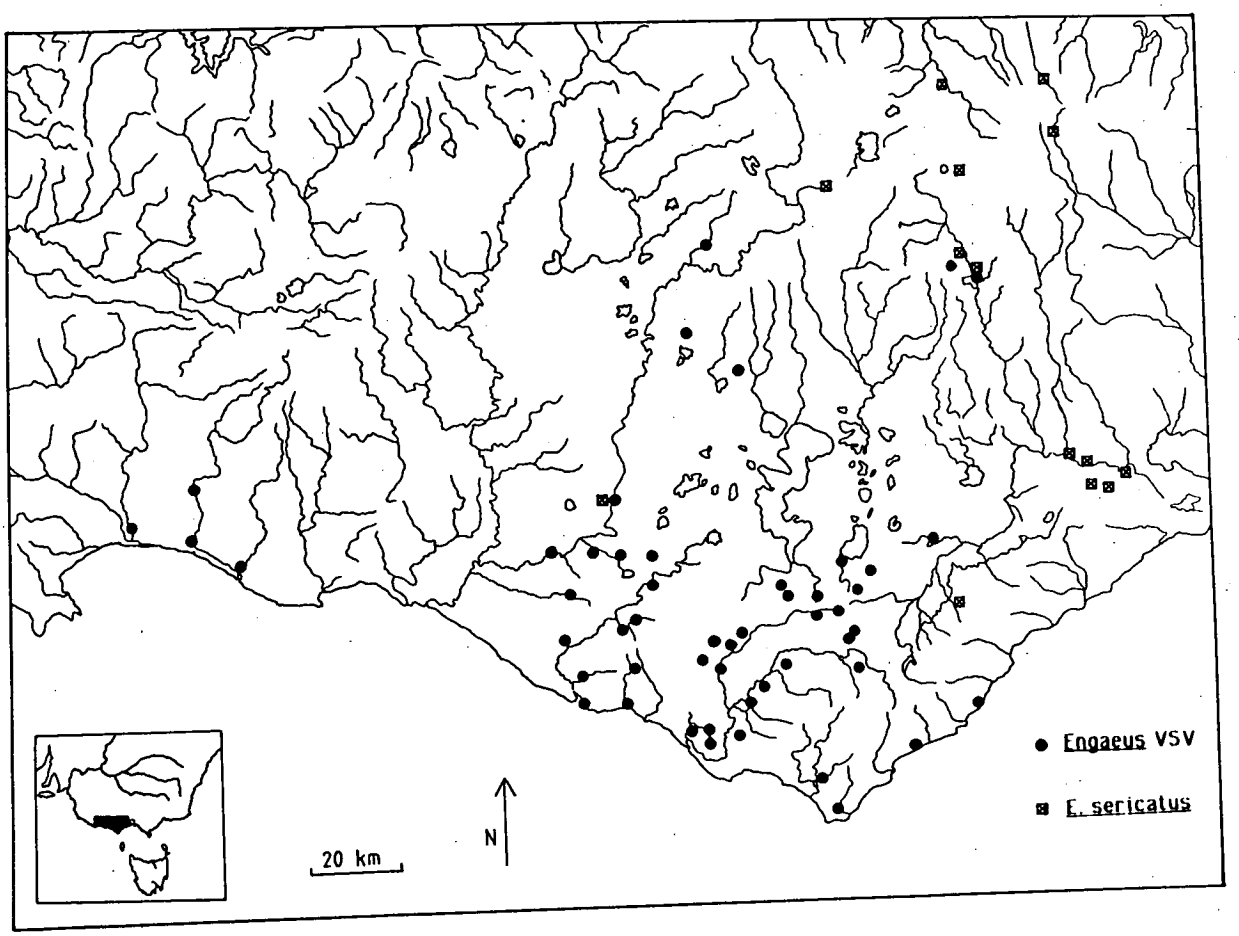


Figure 7: Distributions of 2 species of *Engaeus* in western Victoria (*sericatus* and *Engaeus* VS).

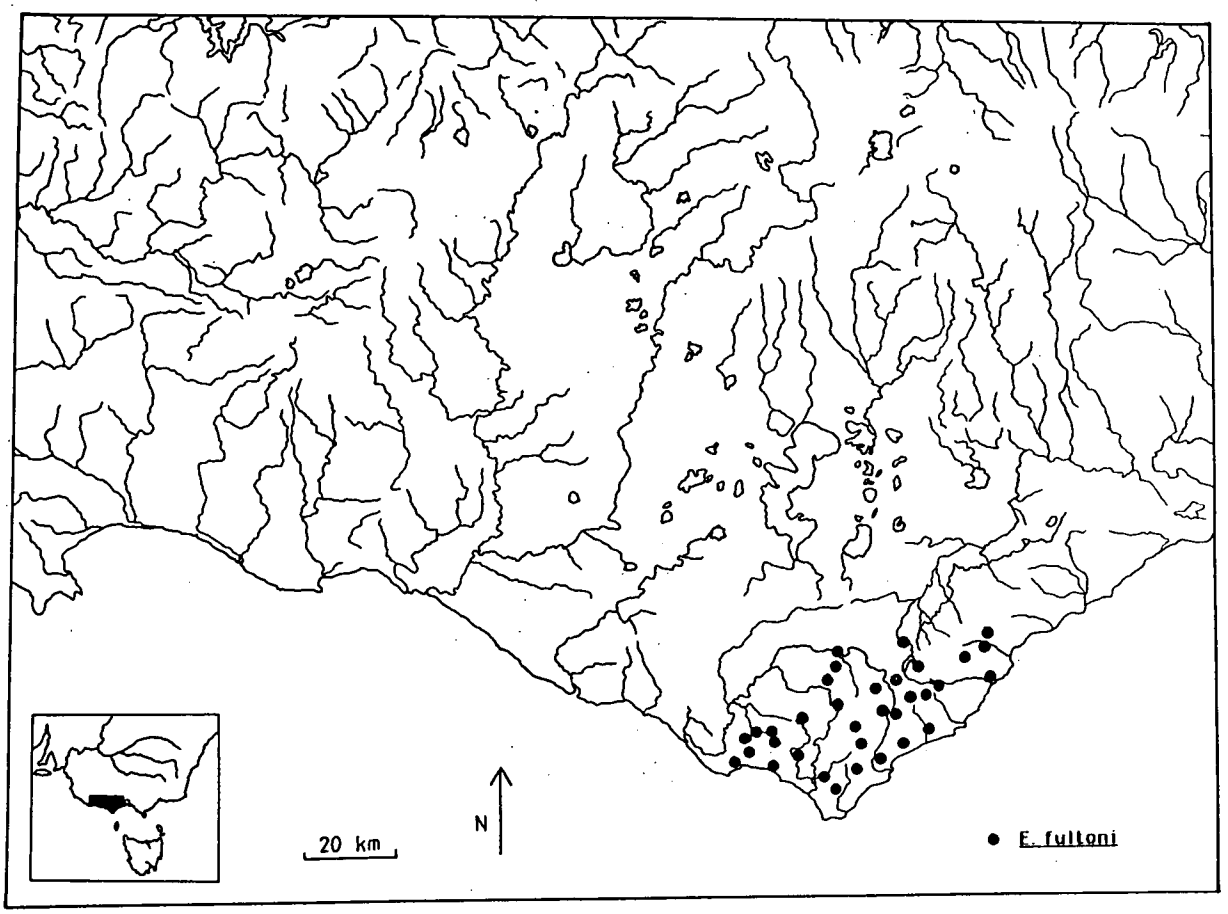


Figure 8: Distribution of *Engaeus fultoni* in southern Victoria.

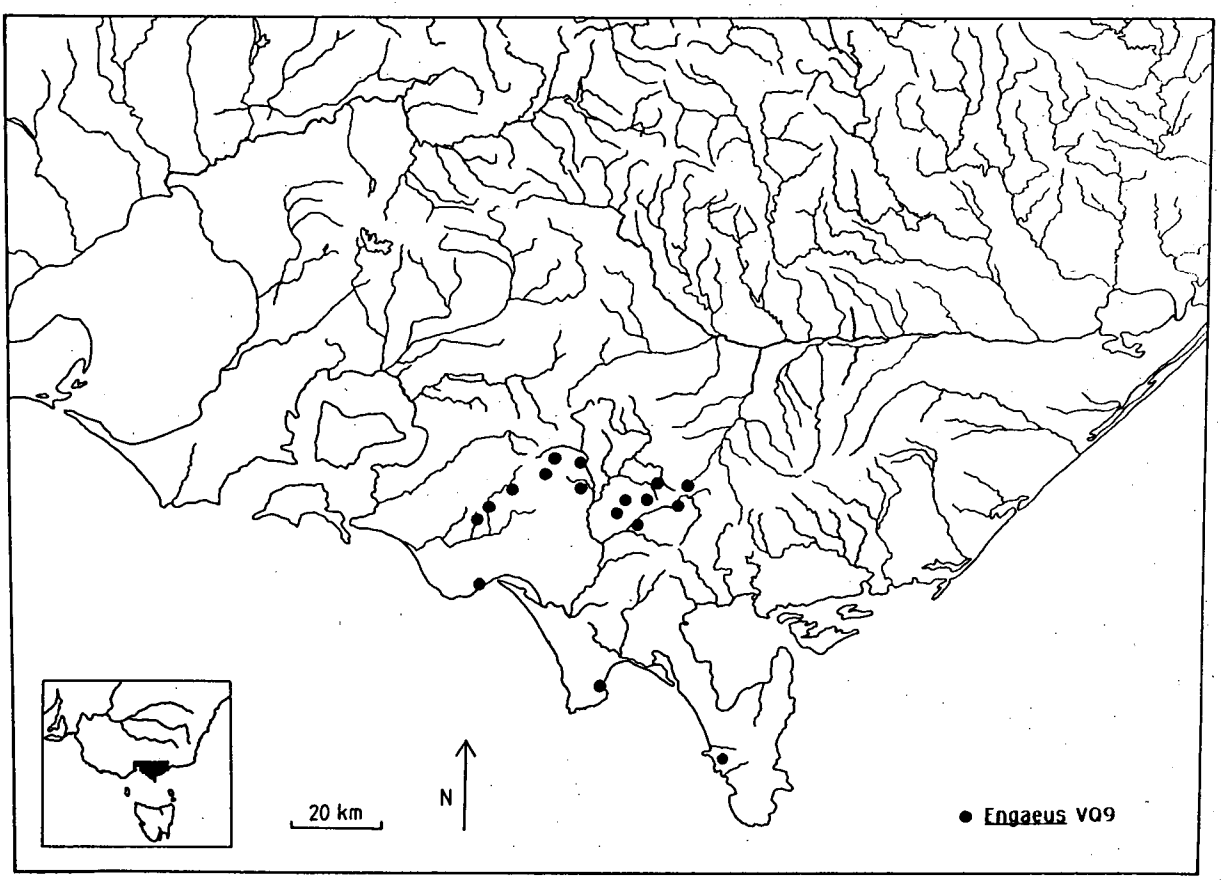


Figure 9: Distribution of *Engaeus* VQ9 in southern Victoria.

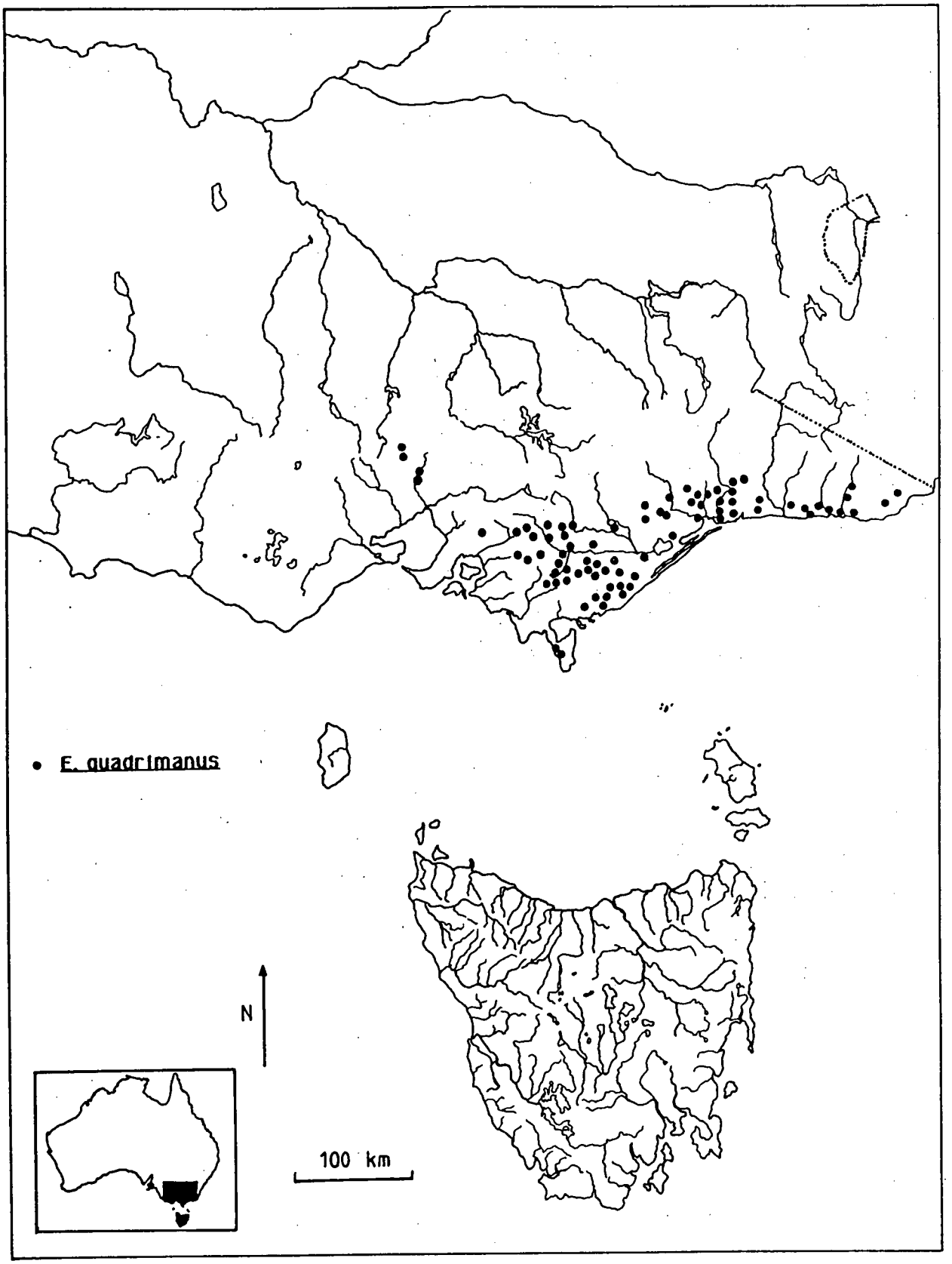


Figure 10: Distribution of *Engaeus quadrimanus* in eastern Victoria.

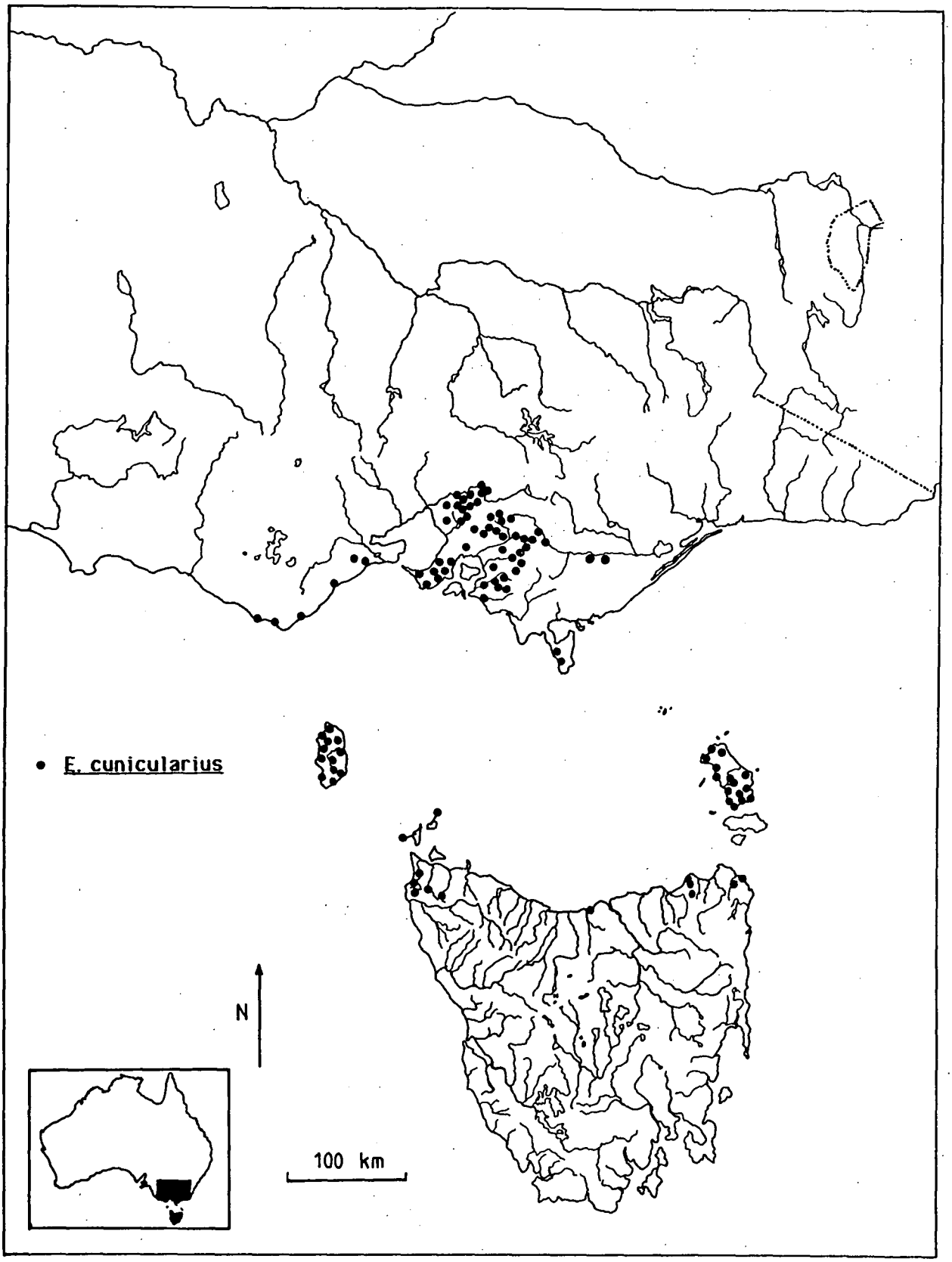


Figure 11: Distribution of *Engaeus cunicularius* in the Bass Strait region of Australia.

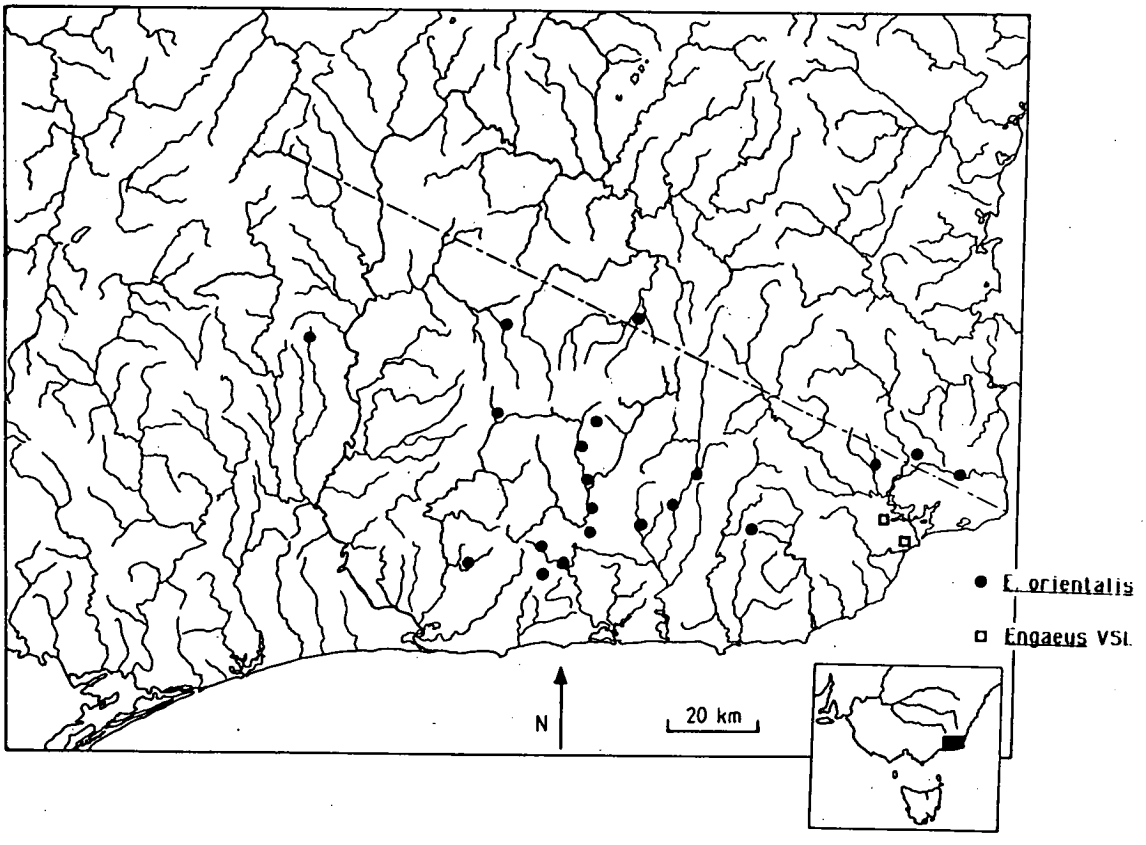


Figure 12: Distributions of 2 species of *Engaeus* in eastern Victoria (*orientalis* and *Engaeus* VSL).

E. strictifrons, *E. laevis* and *Engaeus* TJ (all in Figure 13):

E. strictifrons has been found in the western portion of Victoria and makes an incursion into the extreme south-eastern region of South Australia near Port MacDonnell. Its easterly extension in Victoria is poorly understood; at present it is known as far as Warrnambool.

E. laevis occurs in the north-east of Tasmania and in eastern Victoria. In the latter region it is found on both sides of the Strzelecki Ranges in South Gippsland, and in an isolated cluster of localities in East Gippsland; this disjunct distribution needs to be further investigated.

Engaeus TJ occurs exclusively in western Tasmania where it can be found on Hunter Island and in the extreme north-west, and in a cluster of localities near Macquarie Harbour. The disjunct distribution may well be the result of insufficient sampling.

The *cisternarius* group:

E. cisternarius (Figure 14) has a broad range in north-western Tasmania; its distribution incorporates the southern-most occurrence of *Engaeus* at the Gordon River.

Engaeus TN (Figure 14) has been recorded from two pairs of disjunct localities in Tasmania, one pair near Port Sorell in the central-north, and one pair near Roseberry on the west coast. The region between these localities has been poorly collected.

The *leptorhynchus* group and *Engaeus* TD (all in Figure 15):

E. leptorhynchus is found in north-eastern Tasmania where it occurs in localities within a triangular region bounded by St. Helens, Mt. William and Upper Blessington.

Engaeus TD occurs in north-eastern Tasmania but only at sites associated with northern-oriented drainages, from Pipers River at its eastern extension of its range, to the Ringarooma River.

Engaeus TQ is restricted to a very small geographical range, on Mt. Strzelecki, Flinders Island. It has not been found in Tasmania and is unlikely to be found elsewhere except for other high localities in the Furneaux Group.

Engaeus TA is also known from only a very restricted locality near Lilydale in north-eastern Tasmania.

The *fossor* group:

E. fossor (Figure 16) is widespread in western Tasmania, extending from near Deloraine in the central-north, to Hunter Island in the extreme north-west and south to Gordon River on the west coast.

Engaeus TB (Figure 17) is found predominantly in creek systems of northern-oriented drainages in north-eastern Tasmania, from near Port Sorell to near St. Helens.

Engaeus TBZ (Figure 17) has a more restricted distribution, being confined to the Port Sorell region of central-northern Tasmania.

Engaeus TM (Figure 17) occurs in central-northern Tasmania, south of Port Sorell to as far as Blackwood Creek, near the base of the Great Western Tiers.

Engaeus TF (Figure 17) is restricted to buttongrass plains near Mt. Horror in north-eastern Tasmania.

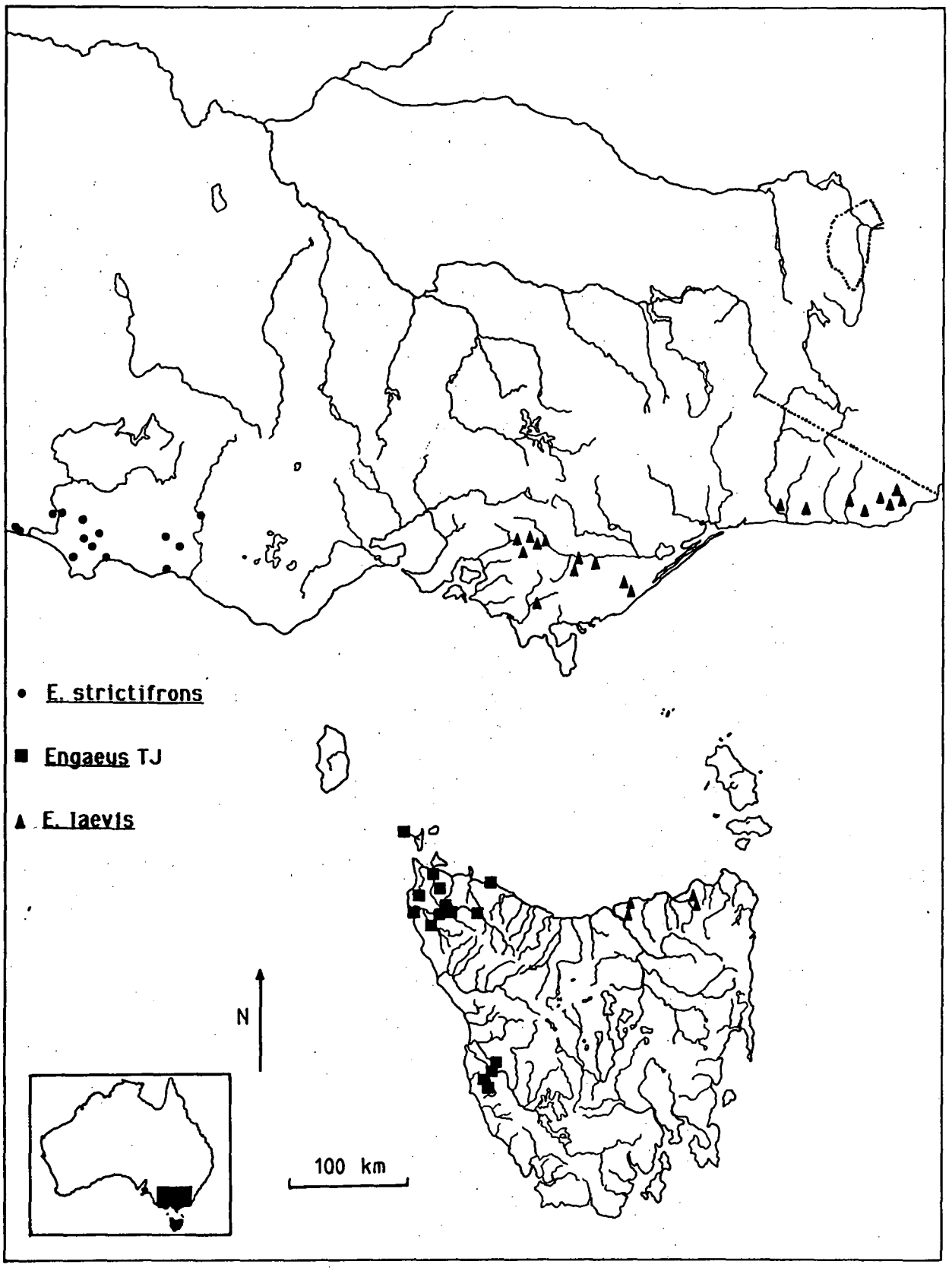


Figure 13: Distributions of 3 species of *Engaeus* in Bass Strait region of Australia (*strictifrons*, *laevis* and *Engaeus* TJ).

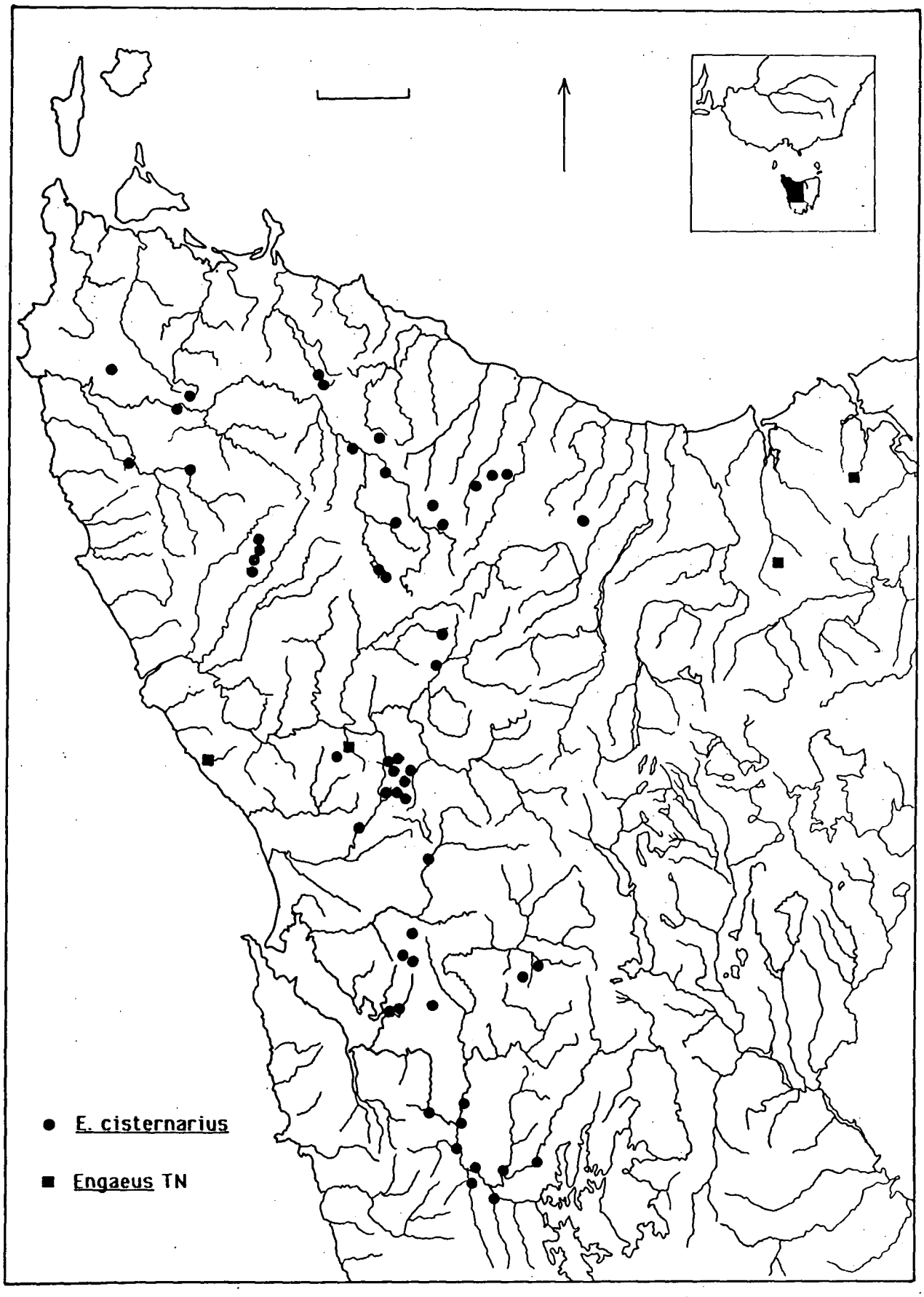


Figure 14: Distributions of 2 species of *Engaeus* in north-western Tasmania (*cisternarius* and *Engaeus* TN).

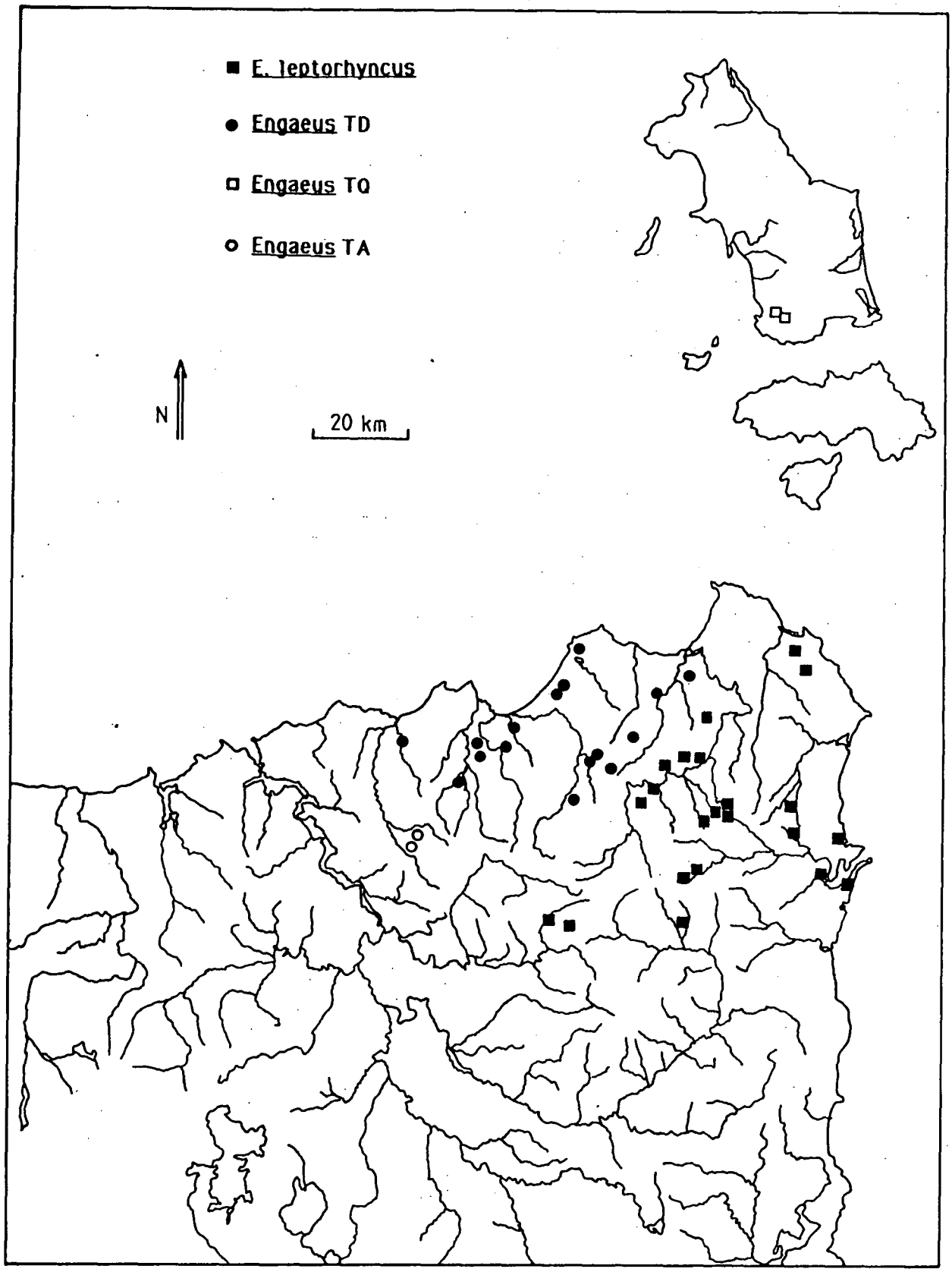


Figure 15: Distributions of 4 species of *Engaeus* in north-eastern Tasmania (*leptorhynchus*, *Engaeus* TA, *Engaeus* TD and *Engaeus* TQ).

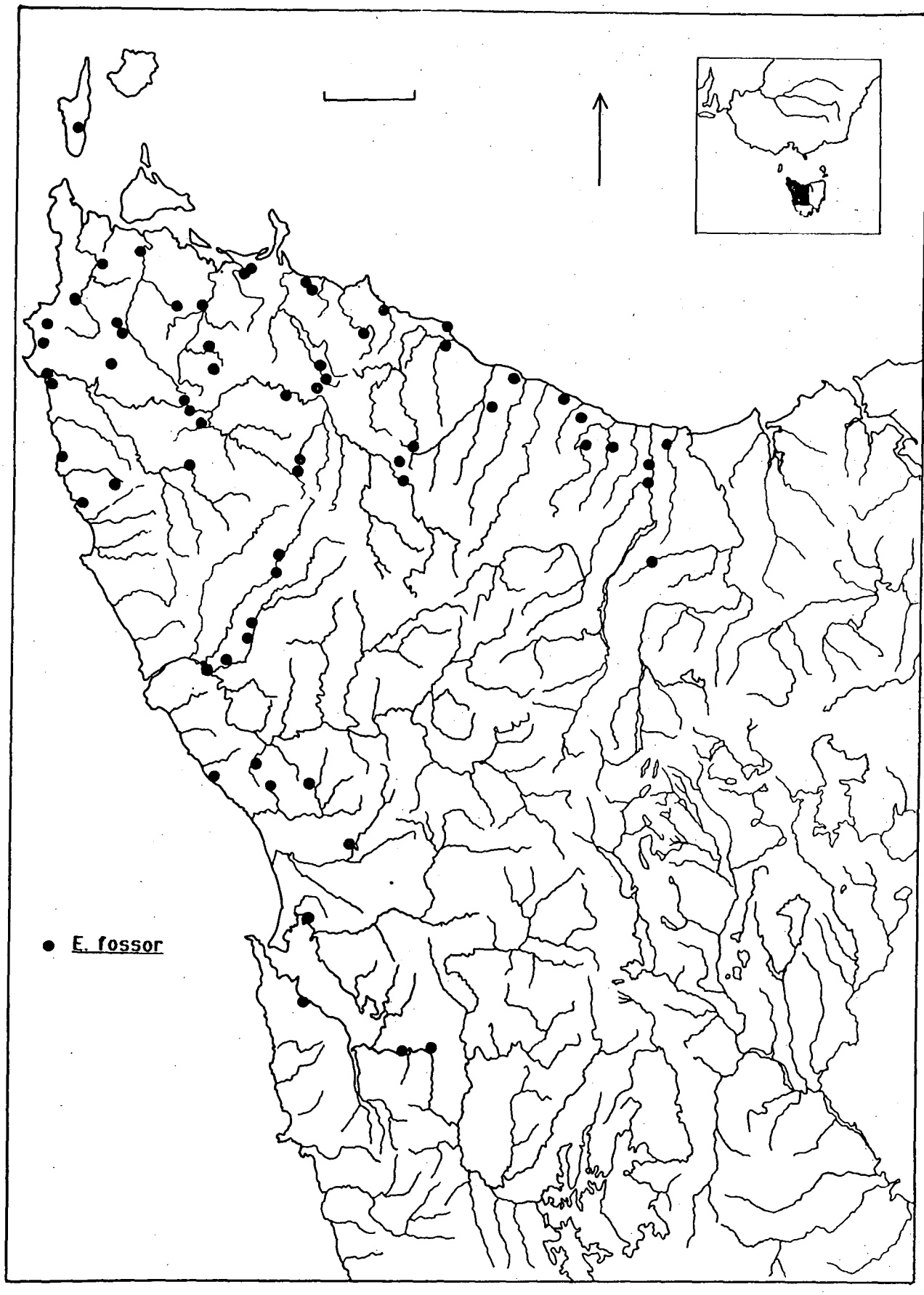


Figure 16: Distribution of *E. fossor* in north-western Tasmania.

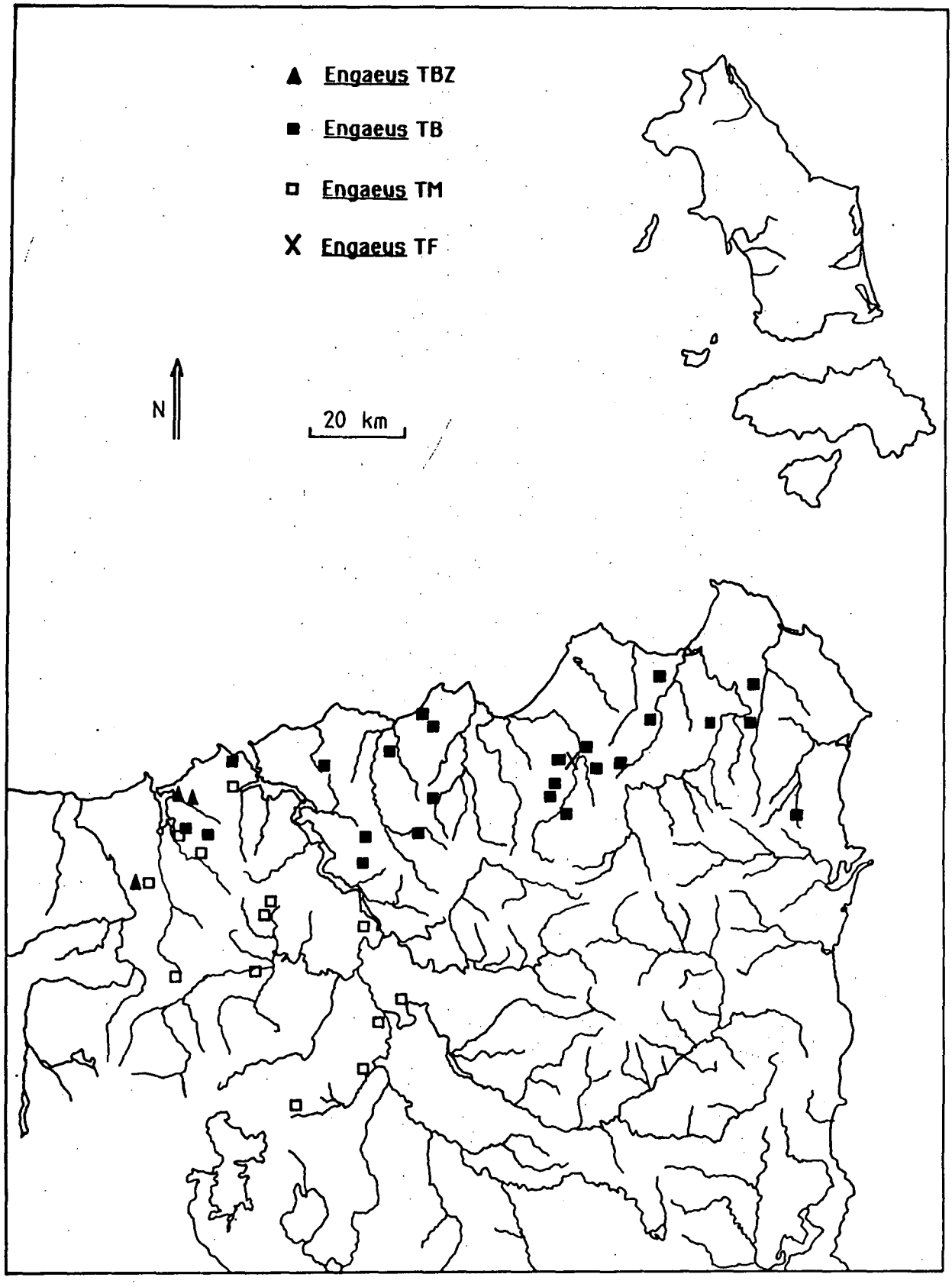


Figure 17: Distributions of 4 species of *Engaeus* in north-eastern and central-northern Tasmania (*Engaeus* TB, *Engaeus* TBZ, *Engaeus* TM and *Engaeus* TF).

E. lyelli:

E. lyelli (Figure 18) has a wide distributional range north of the Great Dividing Range, extending from just west of the Grampian Ranges to near Myrtleford in north-eastern Victoria.

Geocharax sp.:

This genus is found on both sides of the Bass Strait, in north-western Tasmania, on islands in the Hunter Group, on King Island and abundantly in western Victoria, predominantly around the Grampian Ranges, around Portland and in the northern foothills of the Otway Ranges. The occurrence of individuals in South Gippsland represents an intriguing disjunction (Figure 19). In this study it will be assumed that the distributions presented here are those of only one species of *Geocharax*.

Gramastacus (spp.):

The distribution and of this genus is poorly known; one species can be found around the Grampian Ranges; another undescribed species can be found at an isolated coastal region between Wyong and Forster in New South Wales.

Engaewa spp.:

This genus is assumed to be comprised of three species; two of the three previously described species may well be synonymised in the future (*Engaewa similis* and *E. reducta*) and an additional undescribed form has been found from near Walpole (Horwitz, unpublished data). At present the genus is restricted to the predominantly coastal region in the south-west corner of Western Australia.

Tenuibranchiurus spp.:

Species in this genus are found in coastal swamps in the extreme north of New South Wales and south of Queensland. The northerly and southerly extensions of its range need to be investigated. At least two species are known (one is described and one is undescribed; Horwitz, unpublished data).

SUMMARY

A total of 42 species have been included for examination in this study; 34 species of the genus *Engaeus*, 3 from *Engaewa*, 2 from both *Tenuibranchiurus* and *Gramastacus*, and 1 from *Geocharax*. Many of their distributions can be characterized by very restricted geographical ranges, and these ranges highlight regions where endemicity is high. Such geographical regions are the subject of Section 4.5.

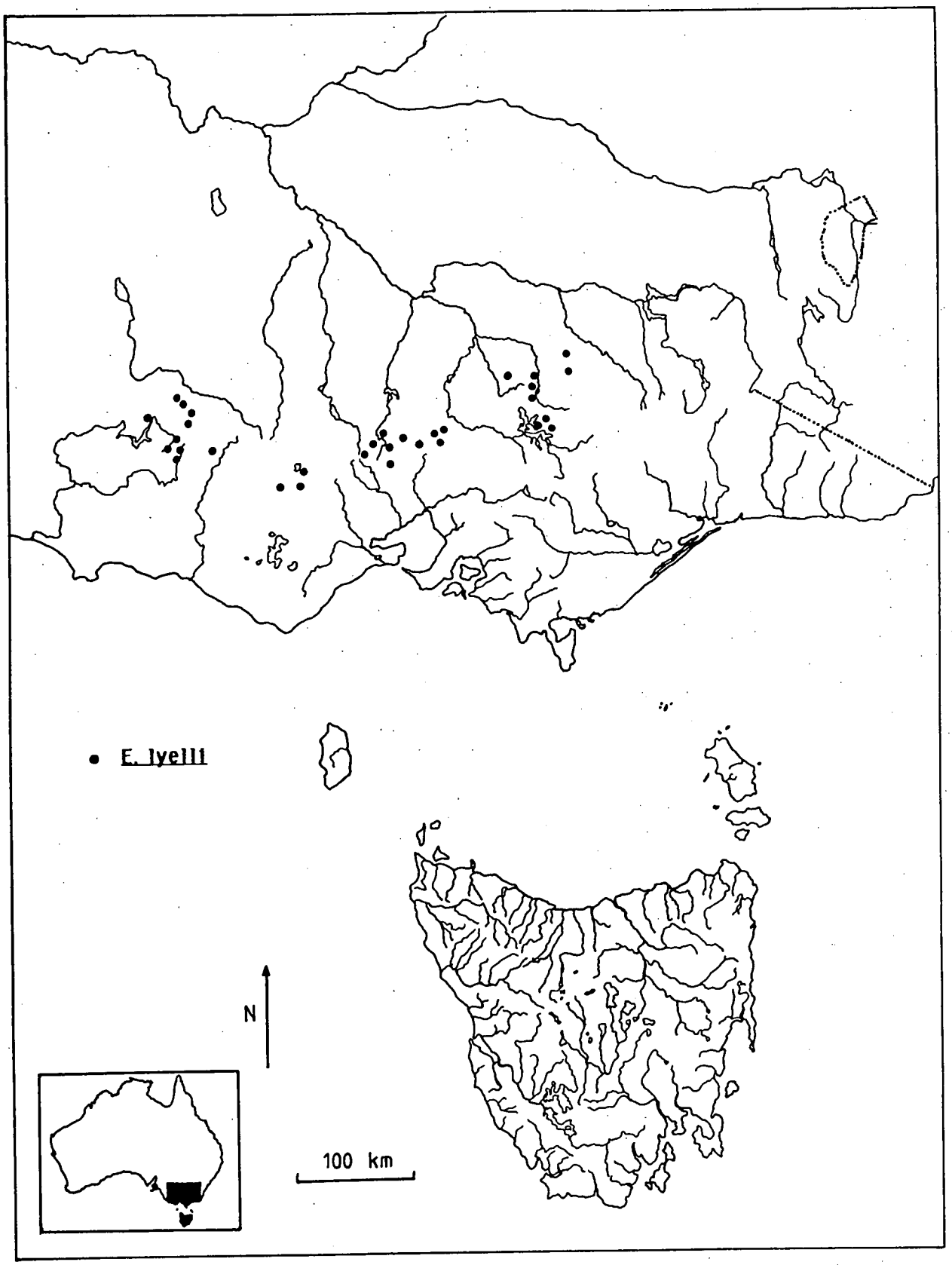


Figure 18: Distribution of *E. lyelli* in Victoria.

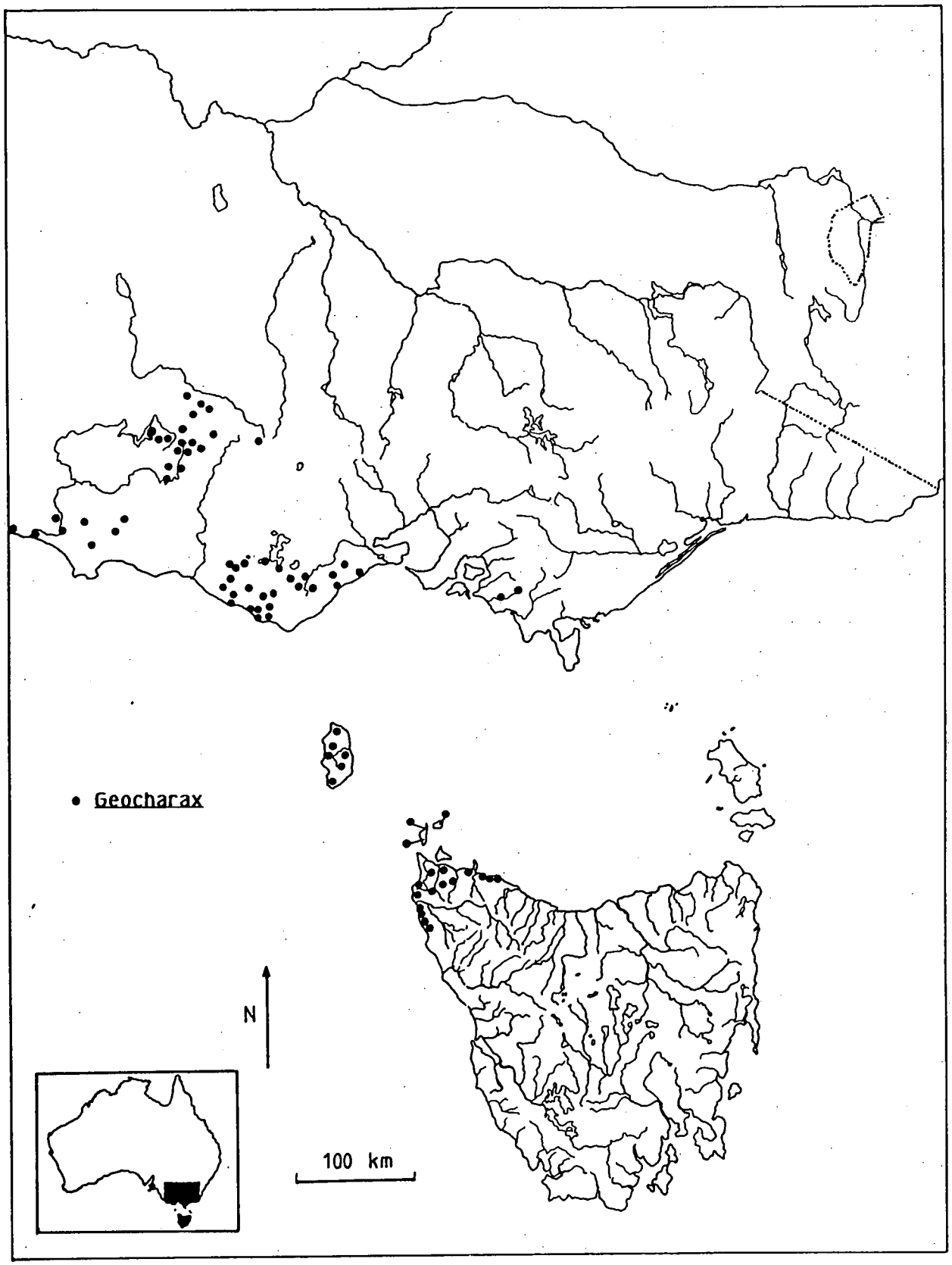


Figure 19: Distribution of *Geocharax* sp. in Victoria and Tasmania.

Section 4.3 DISPERSAL

Certain assumptions on the dispersal rates of freshwater crayfish can be made to give us at least a basic understanding about the capacities of these species to cover large (or even small) distances. We know for instance that crayfish cannot fly (unlike some other freshwater macroinvertebrates), that they do not have a resistant stage or one which will transport them over or through a region of physiological stress to another suitable region and that they do not have a marine phase (unlike freshwater fishes such as some of the galaxiids). Consequently, crayfish are most unlikely to disperse across large areas of desert or ocean. Smith's assertion (1912) that members of the genus *Engaeus* colonized Tasmania by floating in logs across Bass Strait from Gippsland, therefore, cannot be given credence. Due to their size and probably limited abilities to cling for long periods of time, freshwater crayfish are also most unlikely to be transported by organisms across large distances, such as in the plumage of wetland birds. The most likely modes of transport for freshwater crayfish are self-propulsion, either in large bodies of freshwater or terrestrially; as such these organisms should be considered as having a relatively low vagility.

In general the habitat utilization of a crayfish species can give the most indirect information on possible rates of dispersal. For instance crayfish which are capable of occupying type 1a, 1b or 2 burrow habitats (sensu Horwitz and Richardson, 1986) in lowland plains or areas capable of inundation are likely to be given the opportunity to disperse over relatively large distances in a short period of time. This could be possible through the aid of access to permanent or standing waters, particularly during conditions of flood which increase the distances covered by such water bodies, and particularly for juveniles specimens. These individuals may disperse by swimming across the area covered by an inundation or flood, a situation which can be proposed for the broad distributional range of *Cherax destructor* in central-eastern Australia. Alternatively it is at least theoretically possible for juvenile crayfish to become incorporated into the drift fauna for some rivers and streams, again, particularly in time of excessive rainfall; however no records of freshwater crayfish have been made in drift sampling. Evidence to back up these assertions can be provided by the frequent observation during the course of this study of juveniles or small adults amongst submerged vegetation in standing water. Such specimens were collected readily by the use of an FBA net and individuals of species representing the freshwater crayfish genera of *Engaeus*, *Engaewa*, *Geocharax*, *Gramastacus* and *Tenuibranchiurus* were amongst those collected. The most productive period for such collections was in spring (or autumn for the latter genus), and this period presumably coincides with the highest levels of the water-table.

Individuals of species which are capable of occupying type 2 or type 3 burrow habitats in highland areas are much less likely to become incorporated into permanent or standing waters. No record, in either this study or any others, has indicated the occurrence in a net sample of an individual of a species which is capable of occupying a type 3 burrow. The major mode of dispersal for these species is presumably by walking above ground in periods of high humidity. Such activity has indeed been noticed, for instance by Horwitz et al.

(1985a). In fact these authors suggested that the occurrence of burrows with large chambers filled with many juveniles of at least one age-class, and their subsequent release at a large juvenile stage, was an adaptation to a more terrestrial existence where dispersal into permanent water is not possible.

In summary, two possible modes of dispersal of freshwater crayfish can be proposed, the first is by swimming in standing or running freshwater bodies, and the second is by walking. These methods are unsuitable for traversing either oceanic waters or desert areas, and they presumably give the freshwater crayfish fauna, comprising at least these five genera, a comparatively low vagility.

Section 4.4 COMPARISON OF SPECIES DISTRIBUTIONS

The terminology used to categorize distributions is varied and requires some preliminary clarification, particularly since some terms can be confused with the terminology for modes of speciation, and since some of the terms may not be directly applicable to the degree of present understanding of crayfish distributions.

Perhaps the easiest distributions to interpret are those where any two species do not overlap in their geographical range; the term used to describe these distributions is *allopatric* (sensu Mayr, 1942). Different forms of allopatry have been distinguished, but in this thesis only two have been used; *disjunct* distributions are used to describe geographical ranges which are clearly separated, whilst *parapatric* distributions are used to describe abutting or contiguous geographical ranges (Wiley, 1981). In theory disjunct and parapatric distributions are easily distinguished; however occasionally distributions are found which cannot be easily classified. The terms 'abutting' and 'contiguous' appear to be the source of this conflict. For instance do they apply to species whose distributional ranges abut within 1 metre of each other, within 100 m of each other, in separate drainages or all of these? For the purposes of this thesis, the term of 'parapatric distribution' is used to include those distributions which can be separated by an imaginary line on a distributional map, on one side of which is one species, and on the other side is another species, where the width of the line can be quite large (in the range of 100-1000 m for instance) and where the species concerned are capable of occupying the same microhabitat.

Where the geographical ranges of two species do overlap, the embracing term *sympatry* (sensu Poulton, 1903) has been used. In the past this term has been used both to describe species which occur over the same geographical range but occupy different habitats, and to describe species which occur together in the same habitat. In addition the degree to which the sympatry occurs over each species' range needs to be addressed. For freshwater crayfish, only rarely will sympatry between two species be complete, and even on the occasions where it does arise, one of the species is usually restricted to a confined geographical range. In addition, two different types of sympatry have been identified among freshwater crayfish in the genus *Engaeus*, where two species may be found both in the same creek system and in close proximity to each other. The first applies to species that occur in the same portion of the creek, for instance either a lowland or a highland area, and usually these species occupy burrows in different microhabitats of the creek. Such sympatries have been described by Suter and Richardson (1977) and Horwitz et al. (1985b), and for the purposes of this thesis, they will be termed *transverse sympatries*. The second form applies to species which occur in very close proximity to each other but in different portions of the creek, for instance one species may occur in the lowland portion and the other species in the highland portion. Upon close examination of the apparent separation, sites are usually found where both species occur. Consequently, whilst the distributions on a map may suggest parapatry (due to apparent abutting distributions), closer scrutiny reveals a sympatric association. These types of sympatry have not been described for any crayfish fauna, and will be termed

longitudinal sympatries in this thesis.

Having defined these forms of distributional interactions between species, a cross-tabulation can be compiled, indicating the relationship between any one species and all of the other species of the genus *Engaeus*. Tables 1 and 2 give this information for species occurring in Victoria and Tasmania respectively; of course all of the endemic species from one state have disjunct distributions with those from the other state. The tables show species pairs which have disjunct distributions ('0'), parapatric distributions ('P') and sympatric distributions ('TS' for transverse sympatry and 'LS' for longitudinal sympatry). More detailed information can be found in the Habitat Notes for each species in Volume 2. The genera of *Geocharax* and *Gramastacus* have been included in Table 1 since their ranges overlap with those of *Engaeus* species. For similar reasons *Geocharax* has been included in Table 2.

DISJUNCT DISTRIBUTIONS

By far the majority of the distributional interactions between species are disjunct. Without additional information, these disjunctions are difficult to interpret. They may be the result of speciation in separate areas without subsequent dispersal, physiological or ecological selectivity to a certain area or they might even be the result of a former competitive exclusion of one species from a particular area by another species.

PARAPATRIC DISTRIBUTIONS

There is no evidence to suggest that the distributions are caused by specific habitat requirements. Most cases of parapatry occur between species which are closely related to each other, for instance *E. affinis-E. victoriensis*, *E. tuberculatus-E. hemicirratulus*, and *Engaeus* TB-*Engaeus* TM. Notable exceptions to this latter generalization are the *Engaeus* TD-*E. leptorhynchus* interaction, and the *E. strictifrons-E. sericatus* interaction. The implications of this type of distributional interaction can be far-reaching, particularly if it can be demonstrated that a parapatric boundary represents a line which is sufficient to prevent the spread of one species' range into the range of the other. Obviously, these observations need to be investigated further.

SYMPATRIC DISTRIBUTIONS

An important qualification regarding these sympatries needs to be restated, namely that an interaction which has been termed TS or LS does not necessarily mean that such an interaction occurs over the entire overlap of geograhical range, in fact in some cases the ranges of species were disjunct except for a single site where the sympatry occurred. On other occassions, for instance for *E. fossor* and *E. cisternarius*, both longitudinal and transverse sympatries are postulated (the former where *E. fossor* occurs towards the coast and *E. cisternarius* occurs further inland).

Species at sympatric sites, whilst dwelling in burrows within the movement range of each other, clearly occupied different microhabitats within that site. Exceptions to this are found for *E. sericatus* and *Engaeus* VS at their sympatric site, where only one specimen of each species was collected (within a metre of each other) but no ecological information could be collected; similarly, for the longitudinal sympatry between *E. victoriensis* and *E.*

Species	VAFB	VAU	VCY	VF	VQ	VH	VLA	VLY	VO	VPH	VM	VSV	VSN	VSF	VT	VUO	VV	VAFA	VQ9	VRJ	VS	VSL	GEO	GRAM
E. affinis (VAFB)	x	0	P	0	0	P	0	0	0	0	0	0	0	0	P	0	P	TS	0	0	0	0	0	0
E. australis (VAU)		x	0	0	TS	0	0	0	0	0	TS	0	0	0	0	0	0	0	TS	0	0	0	0	0
E. cymus (VCY)			x	0	0	0	0	TS	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
E. fultoni (VF)				x	LS	0	0	0	0	0	0	LS	0	0	0	0	0	0	0	0	0	0	0	0
E. cunicularius (VQ)					x	TS	TS	0	0	LS	TS	0	TS	0	0	0	TS	0	TS	0	0	0	TS	0
E. hemicirratulus (VH)						x	TS	0	0	TS	TS	0	TS	0	P	0	0	0	TS	TS	0	0	0	0
E. laevis (VLA)							x	0	0	0	TS	0	0	0	0	0	0	0	0	0	0	0	0	0
E. lyelli (VLY)								x	0	0	0	0	0	0	0	0	0	0	0	0	0	0	TS	TS
E. orientalis (VO)									x	0	LS	0	0	0	0	0	0	0	0	0	0	0	0	0
E. phyllocercus (VPH)										x	0	0	0	0	0	0	0	0	0	0	0	0	0	0
E. quadrimanus (VM)											x	0	TS	0	0	0	0	0	TS	0	0	0	0	0
E. sericatus (VSV)												x	0	P	0	0	0	0	0	0	TS	0	TS	0
E. sternalis (VSN)													x	0	0	0	0	0	0	0	0	0	0	0
E. strictifrons (VSF)														x	0	0	0	0	0	0	0	0	TS	0
E. tuberculatus (VT)															x	TS	LS	0	0	0	0	0	0	0
E. urostrictus (VUO)																x	0	0	0	0	0	0	0	0
E. victoriensis (VV)																	x	0	0	0	0	0	0	0
Engaeus VAFA																		x	0	0	0	0	0	0
Engaeus VQ9																			x	0	0	0	0	0
Engaeus VRJ																				x	0	0	0	0
Engaeus VS																					x	0	0	0
Engaeus VSL																						x	0	0
Geocharax sp. (GEO)																							x	TS
Gramastacus sp. (GRAM)																								x

Table 1: Interaction of species' distributions in Victoria. See Chapter 3 for species' code and Section 4.4 for explanations.

Species	VQ	TH	TG	TC	TA	TB	TBZ	TD	TF	TJ	TM	TN	TQ	GEO	VLA
E. cunicularius (VQ)	X	0	TS	0	0	0	0	TS	0	TS	0	0	LS	TS	0
E. cisternarius (TH)		X	TS,LS	0	0	0	0	0	0	TS	0	0	0	0	0
E. fossor (TG)			X	0	0	0	0	0	0	TS	0	0	0	TS	0
E. leptorhynchus (TC)				X	0	TS	0	P	0	0	0	0	0	0	0
Engaeus TA					X	0	0	0	0	0	0	0	0	0	0
Engaeus TB						X	P	TS	TS	0	P	0	0	0	TS
Engaeus TBZ							X	0	0	0	TS	0	0	0	0
Engaeus TD								X	0	0	0	0	0	0	TS
Engaeus TF									X	0	0	0	0	0	0
Engaeus TJ										X	0	0	0	TS	0
Engaeus TM											X	0	0	0	0
Engaeus TN												X	0	0	0
Engaeus TQ													X	0	0
Geocharax sp. (GEO)														X	0
E. laevis (VLA)															X

Table 2: Interaction of species' distributions in Tasmania. See Chapter 3 for species' code and Section 4.4 for explanations.

tuberculatus, no microhabitat separation was found at the single sympatric site.

On four occasions of sympatry the species concerned were their closest living relatives. These included *E. urostrictus* and *E. tuberculatus*, *E. affinis* and *Engaeus* VAFA, *E. cunicularius* and *E. quadrimanus*, and *E. sericatus* and *Engaeus* VS. Electrophoretic analysis of the former three pairs of species at their respective sympatric sites, and of other species at many other sympatric sites, revealed at least one fixed allelic difference for each sympatric pair (see Chapter 2). Morphological examination of specimens collected from all known sympatric sites has so far failed to reveal the presence of morphological intergrades. Thus no clear evidence for hybridization between species of the genus *Engaeus* has been found.

Section 4.5 REGIONALIZATION AND ENDEMICITY

The distribution of the five genera (which constitute a monophyletic group) occur within the 'Bassian' biogeographical region (Figures 20A and 20B), with the single exception of the genus *Tenuibranchiurus* (see below). The Bassian region was initially proposed by Spencer (1896) as one of three biogeographical regions in Australia, each of which corresponded to an area of faunal and ecological similarity. With a slight modification proposed by Mackerras (1970; see Figure 20A) along the Dividing Range, the Bassian region corresponds closely to the seasonal, microtherm/mesotherm bioclimatic zone proposed by Nix (1982). The information presented in this Chapter serves to add another group of animals to the list of animals which conform to the Australian biogeographical zones of Spencer.

Species in the genus *Tenuibranchiurus* are found exclusively in northern New South Wales and southern Queensland, occurring in the extensive swamps of the coastal region where they are frequently associated with tall swamp paperbarks of the genus *Melaleuca*, often in the so-called 'wallum' swamps (Riek, 1951). Species of the genus *Engaewa*, although very closely related to *Engaeus* and as such very difficult to separate consistently from it, occur exclusively in the south-west of Western Australia where their distribution in sandy or sandy-clayey heaths and swamps are closely correlated with the occurrence of karri (*Eucalyptus diversicolor*) forests. Both of these regions therefore contain endemic species of freshwater crayfish.

The remaining species belonging to the genera of *Engaeus*, *Geocharax* and *Gramastacus* all occur in the Victoria-Tasmania area. Accordingly, this area will be the focus of all further discussions. The many species in this general area collectively occur over such a variety of habitats, encompassing a wide variety of soils, vegetation and geology, and some are restricted to very small geographical areas. As a consequence of this, and to facilitate a discussion of regional endemicity within this general area, Victoria and Tasmania have been divided into logical (or physiographic) units.

(The discussions will be limited to the above three genera only and will not deal with the presence of species belonging to the freshwater crayfish genera of *Euastacus* and *Cherax*.)

VICTORIA

24 species belonging to the three genera *Engaeus* (22 species), *Geocharax* (1 species) and *Gramastacus* (1 species) occur in Victoria; of these only 17 (all from the genus *Engaeus*) occur exclusively in and adjacent to Victoria. Since the term 'Victoria' is a political one rather than a biogeographical one it is probably much more relevant to examine the physiographical regions of Victoria to determine their respective levels of endemism and to examine them for any relationships between species' distributions and environmental features. The scheme of physiographic regions presented here (Figure 21) is an extension of the scheme given by Duncan (1982) after Jennings and Mabbutt (1977); unless otherwise stated the brief descriptions of each physiographic region are theirs. Climatic information for each region is taken from Lee (1982); soil, vegetation and geological information comes from Rowan (1982)

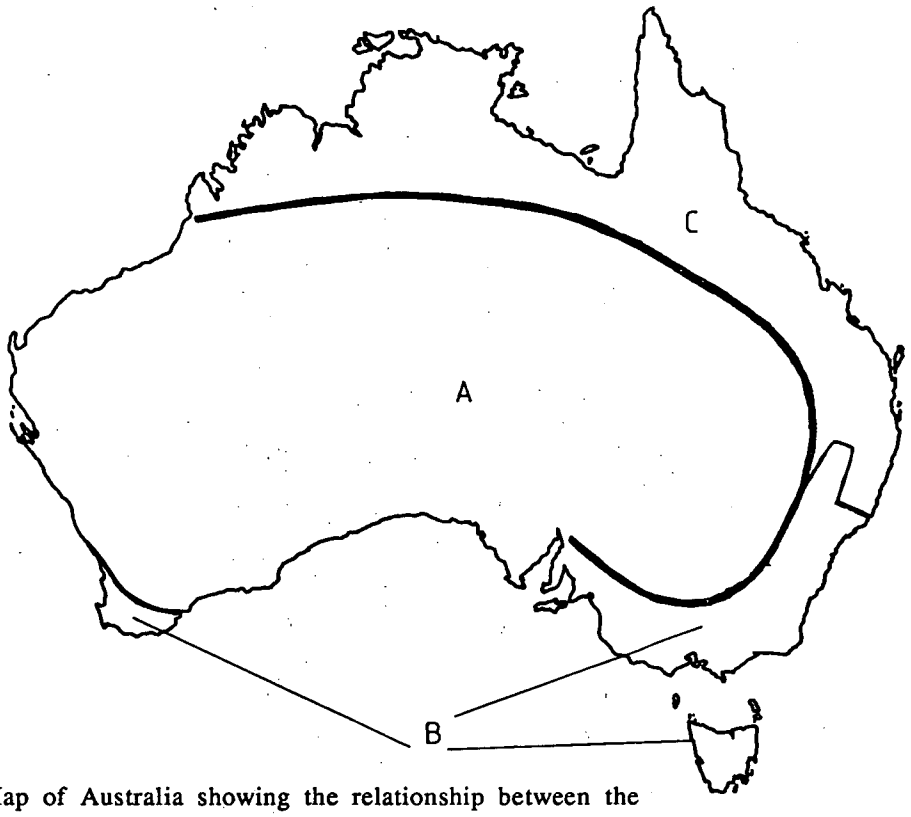


Figure 20A: Map of Australia showing the relationship between the biogeographical regions of Eyrian (A), Bassian (B) and Torresian (C).

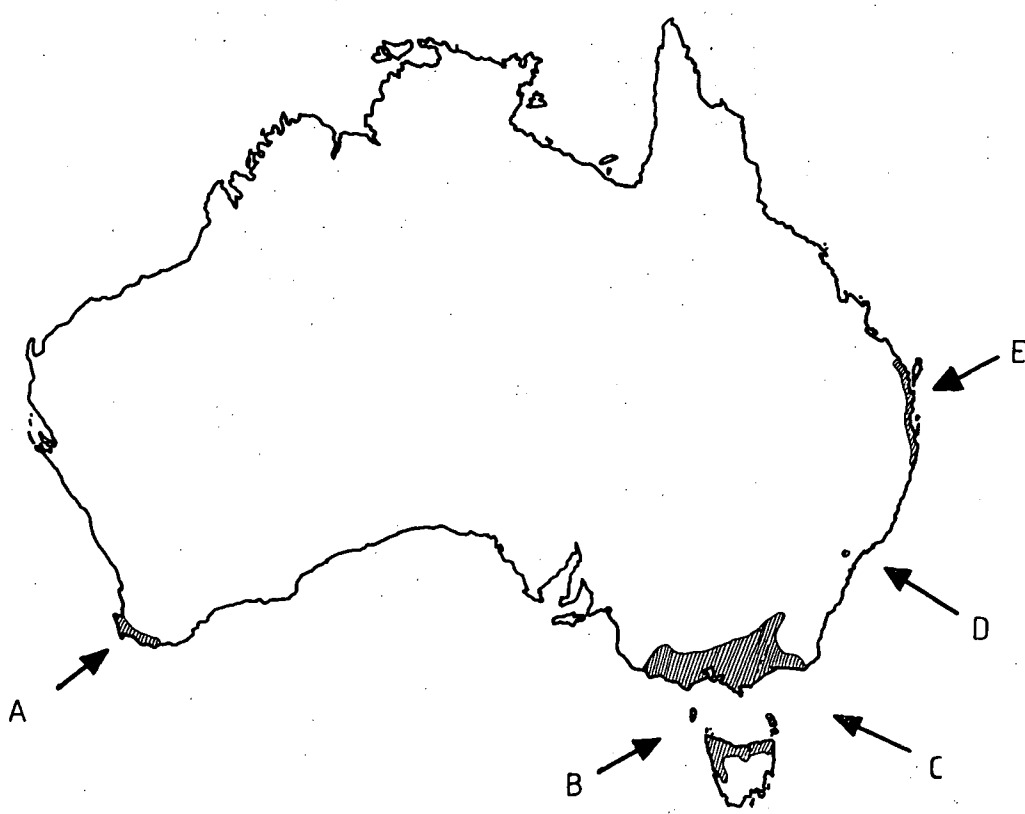


Figure 20B: Generalized distributions of the five genera *Engaeus* (B and C), *Engaewa* (A), *Geocharax* (B), *Gramastacus* (B and D) and *Tenuibranchiurus* (E) (where regions have been highlighted by arrows; see text for more detailed distributions).

Figure 21: Map of Victoria and Tasmania showing the 600 mm isohyet of mean annual rainfall (from Lee, 1982 and data from the Bureau of Meterology, Hobart, Tasmania) and the physiographic regions, where

A = Millicent Plains,

B = West Victoria Plains,

C = West Victoria Uplands,

D = South Victoria Uplands,

E = Gippsland Plains,

F = East Victoria Uplands,

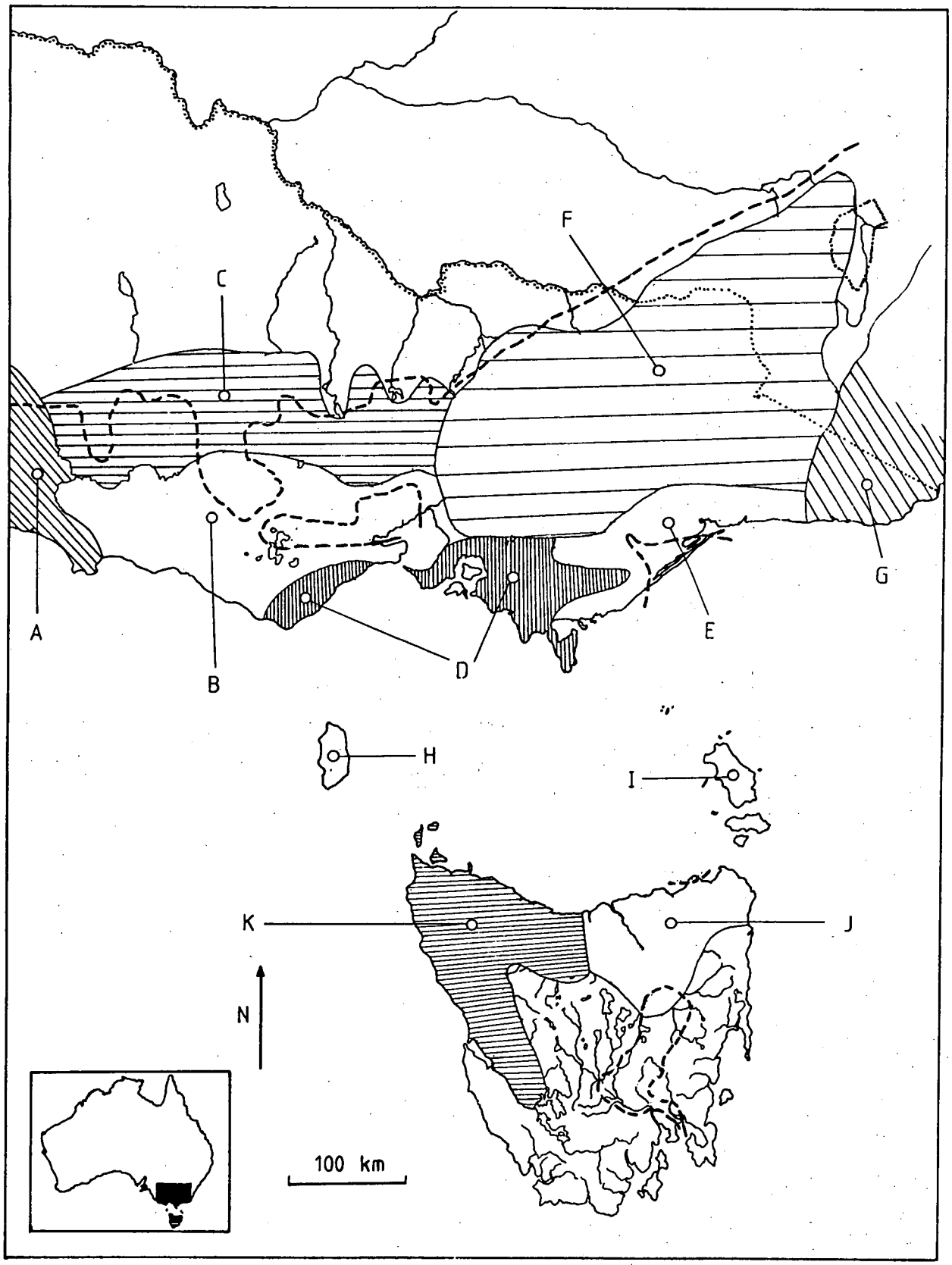
G = Monaro Fall,

H = King Island,

I = Furneaux Group,

J = North-east Tasmania and

K = North-west Tasmania.


A
B
C
D
E
F
G
H
I
J
K
N
100 km

and where possible I have checked this against my field notes taken during 1982-3.

The regions of Victoria are:

MILLICENT PLAIN
WEST VICTORIA PLAINS
WEST VICTORIA UPLANDS
SOUTH VICTORIA UPLANDS
EAST VICTORIA UPLANDS
GIPPSLAND PLAINS
MONARO FALLS

TASMANIA

In the political region that is currently recognised as the state of Tasmania (including King Island and the islands of the Furneaux Group) 14 species of *Engaeus* and 1 species of *Geocharax* are known to occur. Of these, 11 species of *Engaeus* are endemic to the mainland of Tasmania and 1 species of *Engaeus* is endemic to the Furneaux Group. The four regions of Tasmania are shown in Figure 21 and are based on natural boundaries such as the islands of Bass Strait, or on the structural provinces of Davies (1965) within the range of *Engaeus*. Discussions of the physical and biological features such as the soils, geology, climate and vegetation of these regions come from Davies (1965), Pinkard (1980), Richley (1978) and others where mentioned in the text, and where possible have been checked against field notes taken during the period of 1981-3.

The regions of Tasmania are:

BASS STRAIT-KING ISLAND
BASS STRAIT-FURNEAUX GROUP
NORTH-EASTERN TASMANIA
NORTH-WESTERN TASMANIA

MILLICENT PLAIN - parallel dune limestone ridges with intervening swamps; closed karst depressions and young volcanoes in the south-east. Coastal parts of the region have an average annual rainfall of > 700 mm and this decreases inland. The sand plains have either poorly drained acidic sands in closed heath or gleyed podzolic soils in woodland. This region is relevant to this biogeography since it incorporates the species *E. strictifrons*, *Geocharax* and *Gramastacus*; however none of these species are endemic to this region.

WEST VICTORIA PLAINS - mainly on basalt lavas, with many volcanic forms and lakes, partly on weak sedimentary rocks; mainly Quaternary and some Tertiary volcanic rocks, with grey clays, brown podzolic soils and some solodic soils. The plains to the north and west of the Otway Ranges, in open forest, have mottled acidic duplex soils (lateritic podzols); immediately north-west of the Ranges is a sandy plain (composed of Tertiary marine and terrestrial deposits) with swampy, heathy-woodland vegetation. Parts of the entire region receive less than 600 mm, for instance in the area between Geelong and Melbourne, and around the lake region. This may explain the absence of sites where crayfish have been found around these areas. Two species of

Engaeus occur predominantly in this region (*Engaeus* VS and *E. sericatus*); the latter of the two species occurs in abundance in the plains to the north-west of the Otway Ranges. *Geocharax* is abundant, whilst three species of *Engaeus* (namely *strictifrons*, *cunicularius* and *lyelli*) have been found but are uncommon.

WEST VICTORIA UPLANDS - moderately high plateaux and strike ridges, containing the Grampian Ranges in the west and otherwise hilly land with gentle to moderate slopes. The rainfall per annum is > 600 mm except for areas further inland and along a corridor between Ararat and the Grampian Ranges; an absence of crayfish in these areas appears to be correlated with this. The effect of topography on rainfall is exemplified by the Grampian Ranges where rainfall exceeds 1000 mm per annum. The region is characterised by open forest or woodland on yellow sodic or acidic duplex soils. *Geocharax* and *Gramastacus* are both found in the Grampian Range area but not elsewhere in the region, whilst *E. lyelli* is found almost exclusively within the region.

SOUTH VICTORIA UPLANDS - low fault blocks, mainly of tilted and dissected sandstone; granite hills and islands. The region receives an average annual rainfall of always > 700 mm, in fact the rainfall always exceeds > 1000 mm except for

i) a broad, lowland (< 300 m) coastal corridor south-west of the two components of the Strzelecki Ranges (to which *Engaeus* VQ9 is endemic), and

ii) on the Mornington Peninsula.

The freshwater crayfish fauna are abundant and diverse in this region; in the genus *Engaeus* 5 species are non-endemic, of which 3 are common (*E. hemicirratulus*, *E. quadrimanus* and *E. cunicularius*) and 2 are present but rarer (*E. laevis* and *E. victoriensis*). *Geocharax* is present but rare. The region can be further subdivided into the following 4 highland areas (each of which has one endemic species of *Engaeus*): Otway Ranges (*fultoni*), Western Strzelecki Ranges (*phyllocercus*), Eastern Strzelecki Ranges (*Engaeus* VRJ) and Wilsons Promontory (*E. australis*). These areas have rocks of Mesozoic origins and contain tall open forest on friable brown gradational soils (brown earths) and open forests on yellow gradational soils (brown podzols); the wet sclerophyll forests were formerly dominated by very tall mountain ash (*Eucalyptus regnans*) prior to European man's occupation, and currently elements of cool temperate rainforest such as sassafras (*Atherosperma moschatum*) and myrtle (*Nothofagus cunninghamii*) are present but rare in the Otway Ranges, Eastern Strzelecki Ranges and Wilsons Promontory (Howard and Ashton, 1973).

GIPPSLAND PLAIN - terraced plains with sands and gravels. Annual rainfall over this predominantly lowland region is between 600 - 1000 mm, except for the Lake Wellington - Stratford area in which it is below 600 mm and notable for its absence of freshwater crayfish (see Figures 10 and 13). Soils are usually either lateritic podzols (mottled acidic duplex soils), or alluvial (brown soils of uniform texture) and brown podzolic soils (gradational and duplex soils), originating mainly from Quaternary and Tertiary alluvium deposits of sands, silts and gravels. The vegetation consists mainly of woodland swamp species (for instance *Melaleuca squarrosa*). The distribution of *E. quadrimanus* correlates well with the area covered by this

region, as does that of *E. laevis* (in Victoria only).

MONARO FALL - deeply dissected, steeply sloping plateau margin in metamorphic rocks and granite. Four species of *Engaeus* occur but only in the southern portion, in East Gippsland, where the average annual rainfall is over 700 mm and over 1000 mm for many parts of the more mountainous areas. *Engaeus* VSL is endemic to this area where it appears to be confined to the loam and clay plains around Mallacoota Inlet where the vegetation consists of often closed wet sclerophyll forest, often with elements of warm temperate rainforest such as the lilly pilly (*Acmena smithii*), the eastern leatherwood (*Eucryphia moorei*) and the sandpaper fig (*Ficus coronata*). *E. laevis* and *E.quadrimanus* are also found in the coastal fringes of this region, in sandplains or loam and clay plains where the vegetation is predominantly dry sclerophyll, open forest or woodland. *E. orientalis* on the other hand (and as for the very small portion of its range in the East Victoria Uplands) is found in the more mountainous areas (usually < 500 m) where the rocks are Palaeozoic and either sedimentary (sandstones, mudstones, shales and slates) or igneous and metamorphic rocks (such as granites, granidiorites and gneiss) and the wet sclerophyll forests frequently contain elements of warm temperate rainforest on yellow gradational soils (brown podzols).

EAST VICTORIA UPLANDS - dissected high plateau on various resistant rocks; this region is vast and incorporates the area immediately east of Melbourne (the Dandenong Ranges) and slopes north of the La Trobe River from east of Melbourne to East Gippsland to Canberra. (Incorporated within this region is the area of the Australian Alps where high uplands exhibit glaciated features locally and widespread periglacial features; *Engaeus* was not found in these areas). The average annual rainfall exceeds 700 mm and for most areas exceeds 1000 mm; rain-shadow effects appear to occur in the upper reaches of the Tambo and Mitta Mitta Rivers where the average annual rainfall is below 700 mm; this may explain the absence of crayfish of the genus *Engaeus* from this region (see Figure 4, *E. cymus*). 12 species of *Engaeus* occur in this region. *E. cunicularius*, *E. quadrimanus* and *E. victoriensis* are predominantly lowland species and this region therefore does not incorporate a major part of their range; they are found in the foothills in the extreme south-west corner of the region. *E. lyelli* extends its range from the West Victoria Uplands to the lower slopes in the north-west corner of the region; *E. orientalis* is also uncommon to the region (see Monaro Fall). *E. hemicirratulus* is located on the moister slopes of the south-western corner of the region where the soils are brown podzols (yellow gradational soils) usually with wet sclerophyll forest (unless the land has been cleared); these are the same conditions which appear to govern its occurrence in the South Victoria Upland region. The remaining six species are endemic to the region (*E. sternalis*, *E. tuberculatus*, *E. urostrictus*, *E. affinis*, *Engaeus* VAFA and *E. cymus*). Of these *E. cymus* has the widest distributional range where it usually occurs over moderately sloped land, in either dry or wet sclerophyll and open forest on podzols (red or yellow acidic duplex soils) from parent rocks of predominantly Palaeozoic origins. Both *E. cymus* and *E. affinis* can be found at altitudes of over 1 000 m where suitable (deep) soils prevail in wet sclerophyll forests.

BASS STRAIT-KING ISLAND - No endemics are found in this region. The only two species

of freshwater crayfish on King Island are *Geocharax* and *E. cunicularius*, and both occur in abundance in predominantly ephemeral ti-tree swamps or coastal heaths. The soils are podzolic and typical of coarse-textured deposits in coastal areas (groundwater podzols and podzols) or yellow podzolic soils with a duplex profile (Nicholls and Dimmock, 1965) where soils in the swamps have a high coarse-grain component. The occurrence of *Geocharax* along the western side of the Bass Strait and *E. cunicularius* on both large islands in Bass Strait and along the north coast of Tasmania appears to correlate well with the presence of these sandy coastal heaths or swamps in these areas. The average annual rainfall of this region exceeds 700 mm.

BASS STRAIT- FURNEAUX GROUP - Only Flinders Island has been surveyed successfully for freshwater crayfish. *E. cunicularius* almost undoubtedly occurs on Cape Barren Island and a report of crayfish burrows on an island in the Kent Group (N. Brothers, National Parks and Wildlife Service, Hobart, pers. comm.) needs to be substantiated but may well be this species. On Flinders Island two distinct areas can be discerned, namely the ti-tree swamps and heaths on groundwater podzols and podzols or yellow podzolic soils (mainly duplex), as described for King Island and where *E. cunicularius* can be found in abundance, and the frequently coarse-grained soils ('skeletal soils', Nicholls and Dimmock, 1965) from the Devonian granitic parent material on Mt. Strzelecki. This latter area displays 'wetter' vegetation not seen elsewhere on the island, such as sassafras, tree ferns, austral ferns etc. and is the locality of the endemic, highland species *Engaeus* TQ. The average annual rainfall of this region exceeds 600 mm.

NORTH-EASTERN TASMANIA - 10 species of *Engaeus* can be found in this region and 7 of these are endemic. This region can be divided into three areas.

Coastal and Undulating Lowlands: The average annual rainfall of this area is between 600-800 mm. The first portion of this area is a northern coastal fringe of Quaternary origins where the occurrence of sandy coastal swamps correlates well with the presence of the non-endemic *E. cunicularius* (as described above); in addition the distribution of the non-endemic *E. laevis* is incorporated within this area. *Engaeus* TD can be found at some coastal sites, particularly where the soils are yellow podzolic and duplex in profile (for instance at Little Waterhouse Lagoon where the A horizon is sandy and the B horizon has a heavy clay component). *Engaeus* TBZ occurs only on the west side of the Asbestos Ranges in gently undulating lowlands in yellow podzolic soils of a mainly gradational profile.

Tamar Graben: This is the only extensive plain in Tasmania (Davies, 1965). It is occupied by *Engaeus* TM, which can be found as far south as Blackwood Creek at the base of the Great Western Tiers; over this range the average annual rainfall exceeds 600 mm, but further south the rainfall is below 600 mm and this may well result in the absence of suitable conditions for crayfish habitation. Over its range the soils are diverse, but they are mainly either lateritic podzols or grey-brown podzols, with a B horizon which has a heavy clay component and this renders the collection of specimens very difficult indeed. *Engaeus* TM can also be found on both sides of the Dazzler Range in undulating lowlands.

Uplands: The average annual rainfall for this area always exceeds 700 mm and occassionally

exceeds 1000 mm, for instance around Scottsdale. This area contains one portion, the Dazzler and Asbestos Ranges between Port Sorell and the Tamar River, which is characterized by poorly developed soils on Precambrian quartzites and phyllites, where *Engaeus* species have not been found (see Figures 14, 15 and 17). Immediately south of this portion, *Engaeus* TN occurs in wet sclerophyll forest which occassionally contain elements of cool temperate rainforest such as myrtle. This species also occurs in North-West Tasmania.

At the eastern portion of the Uplands *E. leptorhynchus* occurs in burrows on yellow podzolic soils (of either duplex or gradational profiles) in gentle or moderate slopes where Devonian granite forms the parent material, frequently resulting in a soil texture with a coarse, quartzitic component (see Horwitz et al., 1985a); the vegetation varies from buttongrass plains to dry sclerophyll forest to cool temperate rainforest. Only *E. leptorhynchus* has been found to occupy true rainforests in this region.

Engaeus TA appears to occur exclusively in friable orange-brown soils with a heavy clay component and with little differentiation in the soil profiles (krasnozems), with wet sclerophyll forest dominated by *Acacia* and *Pomaderris*. Similarly, *Engaeus* TF appears to be restricted to buttongrass plains around the Mt. Horror region. Both of these species display a habitat specificity; however this may be due to their restricted occurrence and further sites need to be investigated to explore these relationships.

Engaeus TB and *Engaeus* TD are both found in undulating lowlands and highlands in diverse soils but mainly the yellow podzolic soils with either duplex or gradational profiles; their vegetational associations are also diverse, predominantly dry sclerophyll, but at some sites near Mt. Horror these species were found in sympatry in wet sclerophyll forest with elements of cool temperate rainforest such as sassafras.

NORTH-WESTERN TASMANIA - Despite the diversity of the soils and geology in this region, the crayfish fauna is remarkably easy to interpret, in direct contrast to the north-east of the state. Six species can be found, of which three are endemic. Of the non-endemic species, *Geocharax* is found in the extreme north-west corner in the coastal swamps and extensive blackwood (*Acacia melanoxylon*) dominated swamps. Three other species can be found in the latter swamps, including the non-endemic *E. cunicularius* and the endemic species *Engaeus* TJ and *E. fossor*.

The high average annual rainfall of always over 900 mm and often exceeding 2000 mm is said to have resulted in the mosaic of cool temperate rainforest, buttongrass plains and wet sclerophyll forest, each depending upon a particular regime of fire, aspect and soil fertility (Jackson, 1965, 1968). It is this constancy which may well have influenced the broad distribution of the three endemic species (*E. fossor*, *Engaeus* TJ and *E. cisternarius*) so that each species can be found from the Gordon River in the south to the extreme north-west. The latter species appears to be more accomplished at the habitation of higher altitudes and in soils of friable clays (krasnozems) from Tertiary basaltic parent materials; the two former species, on the other hand, are both found in lowland areas.

The non-endemic *Engaeus* TN occurs in cool temperate rainforest around Rosebery.

Summary

The distributions of the genera *Engaeus*, *Engaewa*, *Geocharax* and *Gramastacus* fall within the Bassian biogeographical unit, which can be characterized by cool temperatures and seasonal rainfall. Within this division the genus *Engaeus* is known to occur south of Burrinjuck Dam in New South Wales at sites where the average annual rainfall exceeds 600 mm and where glacial and significant periglacial activity has not occurred recently. In general terms, therefore, the absences of species can be attributed to either insufficient rainfall, constantly high temperatures and/or insufficient or unsuitable soil conditions for burrowing.

In Table 3 the following values are given for each of the Bassian Regions:

i) the total number of species found in the Region ('diversity'),

ii) the number of species endemic to the Region, and

iii) the percentage of endemism.

It shows that the three regions of East Victoria Uplands, South Victoria Uplands and North-Eastern Tasmania contain the highest diversity in terms of species numbers and also high levels of endemism. In terms of the actual geographical area covered by each region, the latter two regions show the highest levels. Both of these regions are topographically and climatologically diverse. On the other hand, lowland regions such as Millicent, West Victoria and Gippsland Plains exhibit low levels of both diversity and endemicity. These findings will be discussed below.

Table 3: The total number of species found in each region, with the number and percentage of these which are endemic to that region.

Region	Total No.	No. Endemic	% Endemism
Millicent Plains	3	0	0
West Victoria Plains	6	2	33
West Victoria Uplands	3	0	0
South Victoria Uplands	11	5	45
East Victoria Uplands	12	6	50
Gippsland Plains	2	0	0
Monaro Fall	4	1	25
King Island	2	0	0
Furneaux Group	2	1	50
North-East Tasmania	10	7	70
Western Tasmania	6	3	50

ENVIRONMENTAL PARAMETERS

Correlations between a species' distribution and the occurrence of one or more environmental parameters need not necessarily imply a habitat requirement by that species. Such habitat requirements can only be established by experimentation after correlations have been

hypothesized; these correlations can then be used as predictors for either an absence or presence of a species.

Very few correlations have been established here. The level of rainfall and glacial activity (= soil depth) appear to be good general predictors for *Engaeus* but again this needs to be tested.

LOWLAND-HIGHLAND DICHOTOMY- THE EFFECT OF TOPOGRAPHY

There appears to be a lowland-highland dichotomy to the distributions of at least some of this parastacid fauna, and this has been demonstrated by the use of physiographic regions within the Bassian unit. In general terms, lowland areas can be defined as those with minimal undulation predominantly below the altitude of 200 m. Lowland species are characterized by broader geographical ranges whilst many highland species are restricted to smaller ranges and as such contribute more to high levels of regional endemicity.

Lowland Species - *Geocharax*, *E. cunicularius* and *E. laevis* are the most important lowland species since they occur on both the north and south sides of Bass Strait. *Geocharax* occurs along the western side, *E. laevis* occurs along the eastern side, and *E. cunicularius* is found along all four sides. In general these species occur in coastal habitats.

Highland Species - *E. urostrictus*, *E. tuberculatus*, *Engaeus* VAFA, *E. phyllocercus*, *Engaeus* VRJ, *Engaeus* TQ, *E. fultoni*, *E. australis* and to a lesser extent *E. orientalis* all have reduced geographical ranges in highland habitats and occur within one region.

The topographical effect (increased number of species with increased diversity of topography; lowland-highland dichotomy) may be related to rainfall. Variations in rainfall associated with a diverse topographical area are likely to produce changes in soil conditions and vegetation patterns, the net effect of which is an increased number of crayfish habitats available. The scheme proposed by Horwitz and Richardson (1986) can be used to exemplify this, where lowland regions or plains carry type 1a, 1b and frequently type 2 burrow habitats, whilst topographically diverse regions carry type 1a, 1b, 2 and usually type 3 burrow habitats. An increased number or microhabitats available allows for the coexistence of more species, and this can be observed frequently in South Victoria Uplands and East Victoria Uplands (Horwitz and Richardson, 1986; Horwitz et al., 1985b).

The situation in Tasmania is more difficult to interpret in this way since there is no clear demarcation between highland and lowland regions. Whilst the topographical effect may indeed apply in Tasmania, it may be masked by the non-conformity of the distributional ranges of some of the endemic species, for instance the vast range of the 'highland' species *E. cisternarius* and the restricted range of the 'lowland' species *Engaeus* TBZ (to take the two extreme examples).

VEGETATION, SOILS AND GEOLOGY

A complex interaction of climate, geology, soils and vegetation are undoubtedly the primary ingredients which influence the present-day distribution of burrowing freshwater crayfish.

In very general terms, each species of freshwater crayfish can occupy a variety of

soil types on a diversity of parent material and often in a variety of vegetational communities, such that any attempt to relate these environmental parameters with species' distributions might well be interpreted as spurious in the best of conditions. For instance species such as *E. fossor* may be found in burrows in cleared pasture, buttongrass plains, ti-tree swamps (coastal or inland), blackwood swamps, wet sclerophyll forest (mixed forest) or in rainforest. The soil texture of the habitats for some species may vary markedly; for instance *E. quadrimanus*, *E. sericatus* and *Engaeus* TB can be found in coarse soils high in sand content, loams, silts or even soils with a heavy clay component. In addition, the occurrence of some species in very restricted localities with apparently uniform environmental conditions may cause the observer to impose an interpretational bias and then hypothesize a set of habitat requirements which are unnecessarily restrictive, and not indicative of the possible variety of habitats which the species could occupy over a broader geographical range. Despite these problems some general points for some species can be made.

E. leptorhynchus, *E. australis* and *Engaeus* TQ are found along the eastern side of the Bass Strait, where they all occur in predominantly wet sclerophyll forests, in soils with a component of quartzitic sands, or even approaching skeletal soils, on parent material of Devonian granite.

E. hemicirratulus is found almost exclusively in type 3 burrows and as such appears to be restricted to brown (friable) or yellow gradational soils, which have a light to heavy clay component; such soils are almost always found in highland regions and the vegetation is typically wet sclerophyll forest (but largely cleared for pasture now).

Finally *E. cunicularius*, whilst occupying a variety of soils and habitats with varying floristics in Victoria, in Tasmania appears to be confined to sandy, coastal swamps and streams which are predominantly of alluvial origins. This apparent habitat specificity is important in the discussion in Section 4.7.

Section 4.6 A HISTORY OF THE BASSIAN REGION

A summary of the current understanding of the climatic events, with their concordant geomorphological and vegetational changes, from 65 million years before present (65 my bp) until present, is given below in order to create an impression of the factors in the past which are liable to have had an affect on crayfish distributions in the Bassian Regon.

TERTIARY (from 64-65 my bp to 1.8 my bp)

Australia underwent a steady northwards drift during the early Tertiary; this northwards drift carried the continent through a range of climatic belts. Gentilli (1961) indicated that climates were moister throughout the Tertiary than they are today. Rainforest vegetation apparently dominated the Australian landscape (Kemp, 1978) although there is disagreement over whether this rainforest was tropical or temperate in nature (Nix, 1982).

In the early Tertiary, Australia was characterized by both widespread humidity and temperatures warmer than those experienced in Australia today (Kemp, 1978) and Beard (1977) suggested that these conditions were humid and non-seasonal during the Eocene. Such conditions are said to be partly the result of warm equatorial currents which could travel large distances without becoming impeded by continents (Frakes and Kemp, 1972).

Kemp (1981) believes that a widespread decline of temperatures occurred in the middle Eocene, and then again in the late Eocene-early Oligocene; this inference is based on floristic changes in deposits in the Gippsland Basin. Whilst a significant drop in the temperature occurred in the southern hemisphere at the Eocene-Oligocene boundary (about 40 my bp), including south-eastern Australia (Frakes, 1978), temperatures may have remained warmer in south-western Australia after this for a short period at least (Hos, 1975). Kemp, (1981) proposed that the circum-polar oceanic current which developed around the Antarctic close to the Oligocene-Miocene boundary was instrumental in reducing the efficiency of meridional heat transport between the equator and the pole, effectively cooling both of the continental systems.

The West Victoria Plains and Millicent Plain region of south-eastern Australia appears to have had repeated marine incursions throughout the Tertiary and even in the early Miocene (Kemp, 1981).

During the Miocene Australia's climate was influenced by a marked increase in the volume of ice in Antarctica and further northward drift; apparently lowered temperatures and an increased dry, anticyclonic circulation resulted (Kemp, 1981). Bowler et al. (1976) suggested that this trend towards aridity in Australia began in the middle of the Miocene, with the first appearance of grasses about this time. Grasslands became apparent in the drier, central part of Australia whilst rainforest persisted in the southern and eastern parts of Australia where year round rains continued (Kemp, 1981). Isolation of the western and eastern floras may have occurred in the upper Miocene. The Antarctic ice sheet underwent a massive expansion between 5 and 6 my bp, and this is said to have produced a major regression of sedimentary basins, increased aridity and a relatively rapid retreat of rainforest vegetation (Kemp, 1981).

Data for the Pliocene remain sketchy. Apparently a marked warming of the early Pliocene seas around 4 my bp (which was possibly associated with a higher level of precipitation and subsequent reappearance of rainforests) followed the severe cooling of the latest Miocene event. This may have been somewhat shortlived, however, being followed by a progressive cooling and drying (Galloway and Kemp, 1981). Bowler (1976) suggested that such wetter oscillations have merely been interruptions to the trend towards aridity, set in the middle Miocene.

QUATERNARY (1.5-1.8 my bp to present)

Most of the information for the Pleistocene comes from pollen analyses provided by various authors; this information is synthesized by Kershaw (1981) and most of what is written below comes from this source.

The Pleistocene was characterized by marked climatic fluctuations. Early and Middle Pleistocene saw three widely separated glacials in Tasmania (Colhoun, 1976).

Ca. 350 000-140 000 y bp: Data from Lake George in New South Wales indicates that this period was punctuated with a series of cold and dry periods alternating with warmer and moister periods; these are interpreted as being glacial and interglacial periods.

Ca. 140 000-65 000 y bp: This period embraces the latter part of the penultimate glaciation (the so-called 'Henty Glaciation'), the last interglacial and the earlier part of the last glacial (the 'Lake Margaret Glaciation'). During the interglacial period conditions are described as being similar to those existing at present; a deterioration of these conditions produced a marked drop in temperatures at the end of the interglacial resulting in a lowering of the sea-levels.

Ca. 65 000-40 000 y bp: This period apparently includes an interstadial during which forest in south-eastern Australia showed a brief expansion phase.

Ca. 40 000-25 000 y bp: Temperatures and precipitation appear to be much lower during this period than they are today, resulting in a development of more open vegetation at some sites in south-eastern Australia.

Ca. 25 000-15 000 y bp: The low temperatures and low levels of precipitation are said to have resulted in a reduction of forested and woodland vegetation in south-eastern Australia. A significant area of what is now Tasmania apparently exhibited 'true alpine' vegetation patterns (around 22 000 y bp; Kirkpatrick, 1986).

Ca. 15 000-10 000 y bp: This is a transition period between the end of the last glacial and the Holocene, where either (or both) an increase in temperatures and an increase in precipitation produced a decline in glacial and periglacial conditions, until ice retreated permanently from Tasmania between 12 000 and 11 000 years ago.

Bowler at al. (1976) and Galloway and Kemp (1981) suggest that the Holocene was characterised by relatively stable conditions compared to the earlier fluctuations. Whilst acknowledging this, Kershaw (1981) indicates that some regions have had quite marked variations over this period, such as the West Victoria Plains, where volcanic soils might have limited vegetation to open woodland and thus masked the climatic variability assessed from other data such as lake levels (Dodson, 1974). There appears to be a general consensus that

conditions in south-eastern Australia were warmer and wetter in the period 8000-5000 y bp than they were at any other stage since the last glacial ended (Bowler et al., 1976; Kershaw, 1981; MacPhail and Hope 1984).

Galloway and Kemp (1981) believe that changes comparable to those of the last 40 000 years occurred repeatedly throughout the Pleistocene and note that throughout the late Cainozoic dry conditions appear to coincide with low temperatures and wet conditions with warmer temperatures. MacPhail and Hill (1983) argue that if conditions which prevailed during early glaciations approximated those during the last glaciation such as the glacial-arid climates and cold-steppe vegetation extending across eastern Tasmania and the then-exposed Bassian Plain to the Adelaide region, then the Bassian Rise would have been a long-term effective barrier against the migration of mesophytes in and out of Tasmania. Its effectiveness to at least some of the Tasmanian fauna may have been similar.

SUMMARY

The topographic factor has been nearly constant throughout the period of Late Tertiary to present. However, in general terms the Tertiary appears to have undergone a longterm trend of decreased temperatures and moisture levels; vegetation changes followed this trend. The Quaternary in contrast appears to be characterized by marked climatic and vegetational fluctuations.

Kershaw (1981) suggests that the present climatic conditions are likely to have been of rare occurrence in the past and that they are likely to be short-lived; in fact Galloway and Kemp (1981) go one step further and state that the present distribution of plants and animals in Australia is a very recent and temporary phenomenon. On the other hand, Nix (1982) concluded that

> "...the major radiations and differentiations of the biota took place long before the Quaternary and indeed for many groups, far back in the Tertiary. Even at the level of speciation, the Quaternary climatic fluctuations may ultimately prove to have no more than a 'cosmetic' effect upon already existing taxa and their major influence may have been one of differential extinction."

The above conflict merely serves as an example of the difficulties involved in interpreting historical factors in biogeographic studies. It would appear that a major problem facing biogeographers is trying to determine whether major radiations took place before the Quaternary, or during the Quaternary. There is, however, no apparent conflict over the overall climatic changes themselves.

BASS STRAIT AND SEA-LEVELS

[For the purposes of the following discussions it is necessary to devise a terminology for the regions currently submerged, which would have been exposed under conditions of lowered sea-levels. Thus three 'grabens' are recognised, namely the 'Bassian' (where the waters of the Yarra system and Tamar system joined), the 'La Trobe' (collecting the waters of the La Trobe River) and the 'Western' (collecting the waters of the southern flowing rivers of western Victoria).]

Early during the Tertiary Antarctica and Australia apparently began parting, a process which created the elliptical, NW-SE trending graben called Bass Strait about 65 my bp (Griffiths, 1971). Since then climatic factors have resulted in fluctuating sea-levels which have either flooded or exposed Bass Strait and hence either isolated or connected Tasmania from the remainder of Australia. An examination of the literature may help to understand the biogeographical implications of such events.

Bowler (1982) depicts the state of the Bass Strait region diagrammatically for the period approximately 20 my bp, showing that the Bassian Graben is inundated, as is the La Trobe Graben, and that these two 'seas' are separated by a land bridge along the eastern side of Bass Strait; this configuration is apparently repeated during the Pliocene (Williams, 1974a) when Bass Strait was connected by an eastern land bridge passing through Flinders Island to Victoria.

These assertions are supported by Banks (1965), who suggested that Bass Strait was probably open during the Miocene, closed during part of the Pliocene and open at some stages but closed at other stages during the Pleistocene.

Galloway and Kemp (1981) claim that there is evidence that sea-levels were low in relation to the continent for substantial periods in the Late Tertiary and that it has been the rule rather than the exception for Tasmania and Australia to be connected during the last few million years. They suggested that the approximate extent of the land during the numerous glacial low sea-levels of the Pleistocene is indicated by the present-day -200 m isobath.

It appears that the height of the sea-level as it exists now has only been matched once before since the penultimate glaciation (the Henty Glaciation), during the last interglacial period when sea-levels probably fluctuated between the present-day level and the -40 m isobath; these fluctuations would have meant the severing of connections between Tasmania and the mainland of Australia. These connections were progressively reestablished since the onset of the last glaciation (about 75 000 y bp).

Rawlinson (1974), using a time curve for the fall and rise of sea-levels over the last 35 000 years provided by Milliman and Emery (1968), estimated the time of exposure of land links between the mainlands of Victoria and Tasmania and the islands around them. King Island was hypothesized to have last been connected to Cape Otway in Victoria 14 750 y bp and was isolated from the Hunter Group and Tasmania 11 750 y bp. The Furneaux Group was isolated from Wilsons Promontory 12 750 y bp and from the north-east of Tasmania 10 000 y bp. By extrapolation, Hunter Island became isolated from the mainland of Tasmania about 7 000 y bp. Therefore the last severing of the land bridge between 'Tasmania' and 'Victoria' took place around 12 000 - 13 000 years ago.

It is important to note that Rawlinson's assessment, and that of others who talk in terms of 'land bridges', directs workers to examine the links or bridges themselves for dispersal routes, as if dispersing organisms travelled along the tops of such ridges. Whilst this may be so for terrestrial organisms such as reptiles, those organisms which are confined at least in part, if not totally, to freshwater systems probably would not have travelled along these

routes at all. The implications of this observation will be discussed in the next section.

Galloway and Kemp (1981) also comment on the role of the Nullarbor as a barrier between the south-west and south-east of Australia, stating that the lowered sea-level during glacial times probably exposed a 200 km-wide belt of different ('sandier?') soils on what is now the continental shelf. They went on to suggest that ample corridors for the coastwise movement of psammophilous biota must have existed throughout the Pleistocene.

In summary, it appears that the Bass Strait has been flooded and successively exposed on many occasions since the Miocene, but the most evidence comes for the period since the penultimate glaciation. Thus, there is the potential for Bass Strait to provide repeated vicariant events on the fauna of either Tasmania or Victoria, particularly the members of that fauna that have low vagility, by allowing, at least in theory, dispersal routes for 'slow' migrations between the two areas on more than one occasion. The problem is therefore whether conditions in Bass Strait itself were suitable for the dispersal of individuals which were otherwise incapable of dispersing over large distances in a short period of time.

Section 4.7 SYNTHESIS

In order to understand the processes which produced the current distributions of species we need to have some sort of understanding of the phylogeny or the genealogical relationships between species. For this purpose the results of Chapter 3 will be used. The dendrogram given in Chapter 3 and reproduced here (Figure 22) depicts clusters of species in groups which are more closely related to each other than they are to other species; it is these groups which form the fundamental units of the tree since it was seen to be much easier to recreate phylogenies between closely related species than it was to do so between more distantly related species. Closely related species probably share a relatively recent ancestor, in fact it is probable that the less the divergence between species (in either genetic or morphological terms) the less the divergence time for the species from the same ancestor. Each hypothetical ancestor is labelled in Figure 22. (It is important to reemphasize that the dendrogram which depicts phylogenetical relationships does not have an evolutionary time scale; it does not show relative times of divergences from ancestral species, merely the presence of these ancestral species). Due to the availability of more substantial evidence for both the phylogeny of recent species and the recent climatic, geomorphological and vegetational effects, the best way to interpret possible mechanisms for the speciation and spread of all the species of crayfish is to examine recent species and conditions, reconstruct a scenario for speciation and hypothesize a recent speciation event. By doing this for recent species we at least have models to apply to more distantly related species.

Another major problem in examining Bassian distributions is the determination of the effectiveness of the Bass Strait as a barrier to the passage of crayfish from Tasmania to Victoria or vice versa.

Consequently, a well known group with a recent history (a group of closely related species) has been selected below to examine the mechanisms of speciation and spread of species in the genus *Engaeus*. (An unfortunate side effect of this approach is the discussion of speciation events in the reverse of the order in which they took place).

It must be stressed that despite all attemps to include as much substantive information as possible, all of the proposed events are extremely speculative in nature and they await rigorous investigation, using at least a more exacting phylogenetic technique.

THE *cunicularius* GROUP

E. cunicularius

The 'circum-Bass Strait' distribution of *E. cunicularius* is given in Figure 11, where it occurs in the seven disjunct regions of near Cape Otway, South Gippsland, Flinders Island, north-east Tasmania, central-northern Tasmania, north-west Tasmania and King Island, and it has been found in type 1b or 2 burrow habitats in lowland, mainly sandy coastal areas. Assuming that its capacity for habitat occupation was the same in the past as it is now (predominantly coastal; see concluding remarks of Section 4.5), and according to the dispersal capacities hypothesized in Section 4.2, then the most likely explanation for its present day distribution is as follows:

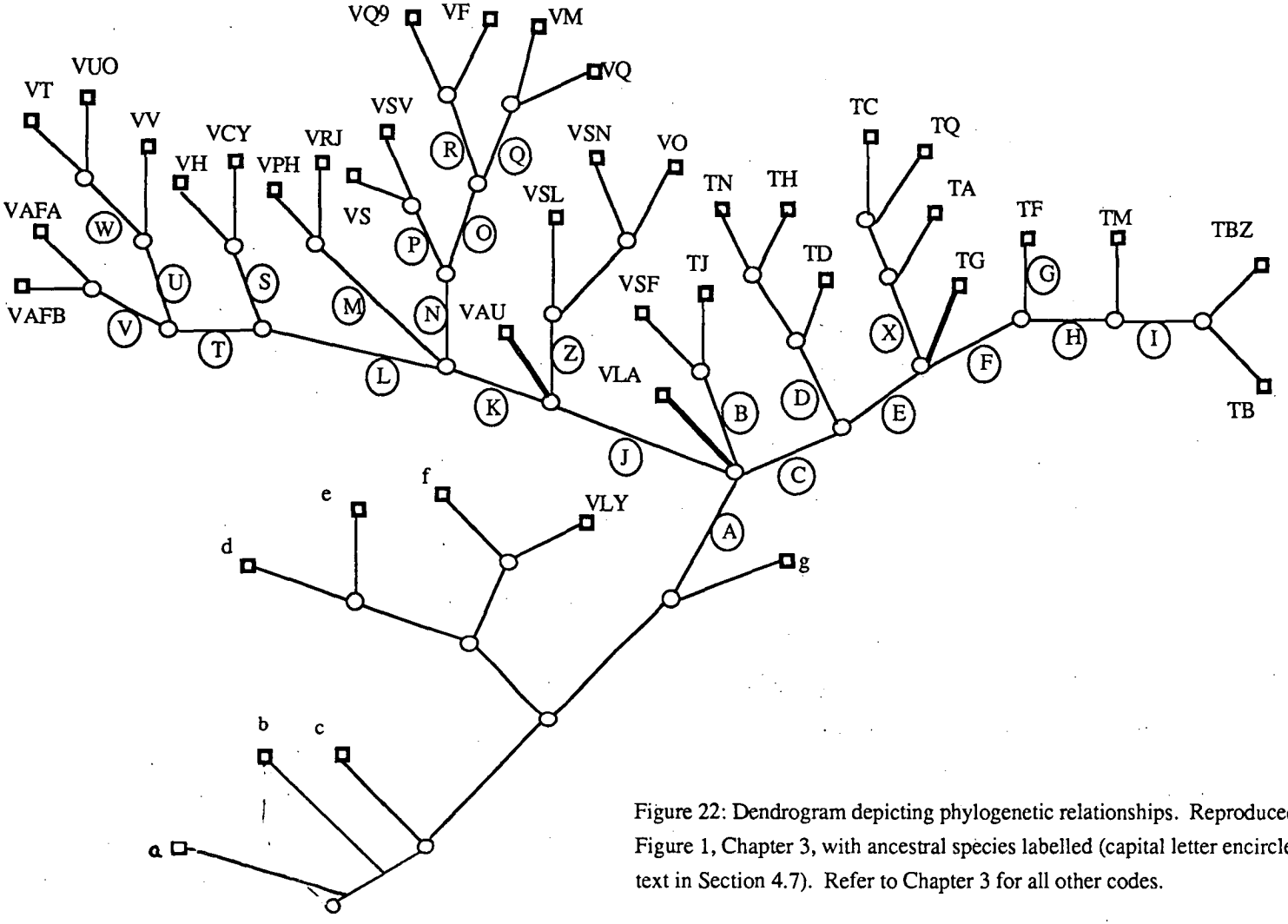


Figure 22: Dendrogram depicting phylogenetic relationships. Reproduced from Figure 1, Chapter 3, with ancestral species labelled (capital letter encircled; see text in Section 4.7). Refer to Chapter 3 for all other codes.

During glacial periods, when sea-levels were lower than they are now, this species was resident in the central Bass Strait region, say at a point on what is now the sea floor somewhere between Wilsons Promontory, Cape Otway and King Island. The conditions here would have been sandy and coastal, and whilst not receiving vast quantities of rainfall, moisture levels close to the coast would have been sufficient to maintain a water-table for psammophilous populations at even the driest times. As sea-levels began to rise, assuming that they did not rise quickly, but at a rate of no more than 1 m in 100 years, populations were gradually pushed back, always staying in front of the oncoming seas by following creek systems or drainage patterns, thereby creating a system of populations radiating out from a single point. This hypothesized process is depicted in Figure 23; under this scheme the sea would have entered Bass Strait from between King Island and Cape Otway, gradually and effectively splitting the initial population.

Some evidence for such a mechanism comes from intraspecific electrophoretic variation (see Chapter 2) and inferred genealogies, since populations from Flinders Island and north-east Tasmania are identical and closely related to that of north-western Tasmania; these populations all exhibit the same unique allele, in distinction to the Victorian populations. In addition these populations all exhibited lower levels of heterozygosity compared to those populations sampled from the Victorian mainland; this suggests that the Tasmanian populations may have passed through a bottleneck (see Chapter 2). The most divergent populations electrophoretically were those from Cape Otway and north-western Tasmania; clearly the analysis of populations from King Island in this study would have been enlightening. The region with most of the intraspecific morphological and electrophoretic variation is found on Mornington Peninsula and in South Gippsland, where presumably regional populations were capable of interbreeding and exchanging uniquely developed characteristics.

Without the aid of fossils or precise molecular data it is very difficult to infer a time during which the above radiations occurred. According to Section 4.6 it is understood that sea-levels as they exist now have been attained on only one other occasion since the penultimate glaciation, and this was probably around 120 000 y bp. If the dispersal of *E. cunicularius* is the result of one complete rising of the sea-level, then it either occurred prior to 120 000 years ago, or much more recently, between 20 000 and 10 000 years ago. Judging by the apparent lack of electrophoretic divergence between populations in Tasmania, particularly between those in north-east Tasmania and Flinders Island, it is tentatively suggested that the latter period saw the dispersal of this species. Whilst exactly the same methods for dispersal are proposed for the other two trans-Bass Strait species (*Geocharax* and *E. laevis*; see below), unfortunately no electrophoretic data were collected for these species in Tasmania and therefore no supporting data for this dating approach can be given.

The 'lowland spread by coastal advance' hypothesis is central to what follows in this section. An interesting implication of the hypothesis is that one species can potentially give rise to more than two daughter species. For instance if the populations of *E. cunicularius* in each of the seven disjunct regions were to maintain themselves without becoming extinct

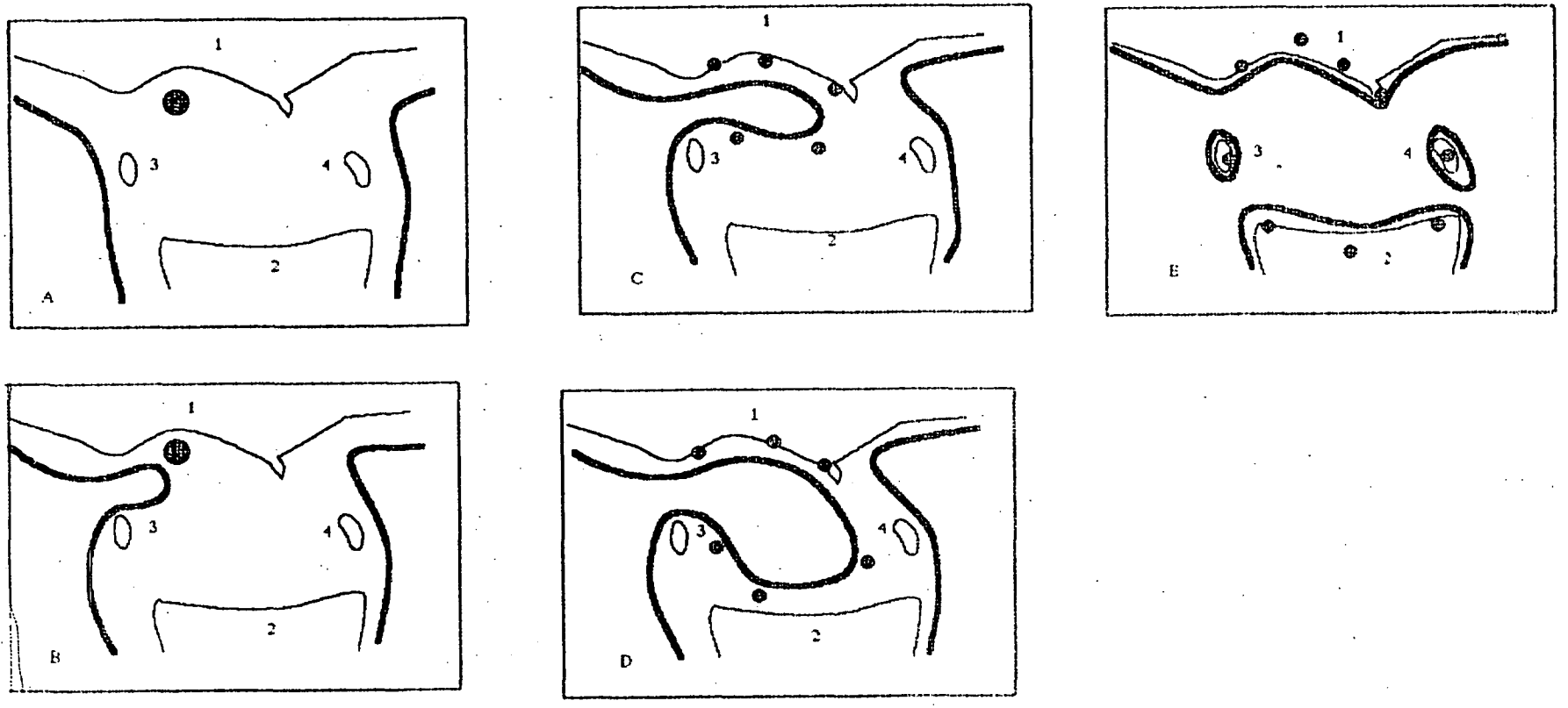


Figure 23: Schematic representation of the Bass Strait region, showing the incursion of the sea through the King Island-Cape Otway pass with time (A-E) during a post-glacial period, and its effect on the hypothetical coastal population of *E. cunicularius*, where the black solid line represents the coastline, the thin line represents a schematic outline of the coastline as it exists today and the hatched circles represent populations of crayfish, and where

1 = Victoria,

2 = Tasmania,

3 = King Island and

4 = The Furneaux Group.

and without interbreeding, then a possible 7 daughter species could result through the agencies of random mutation and reproductive isolation over a certain period of time.

In order to examine some more of the implications of this hypothesis, it is necessary to go further back in time to look at the ancestors of the species in the *cunicularius* group. According to the phylogeny presented in Figure 22, *E. cunicularius* has a sister species *E. quadrimanus* and they share a common ancestor ('ancestor Q'); ancestor **Q** had a sister species, 'ancestor **R**', and ancestor **R** gave rise to two species *E. fultoni* and *Engaeus* VQ9. Ancestors **Q** and **R** themselves are preceeded by ancestor '**O**' who had a sister species '**P**'. **O** and **P** were preceded by the group's ancestor '**N**'.

Ancestor **N**

It is proposed here that ancestor **N** was widespread in the coastal lowlands along the Victorian coast, in areas adjacent to the West Victoria Plains, Bass Plains and Gippsland Plains. As sea-levels rose the species was split into a western Victorian component (**P**, which subsequently gave rise to *E. sericatus* and *Engaeus* VS) and a central-eastern component **O**.

Ancestors **O** and **R**

Ancestor **O** was forced to retract as coastal conditions and sea-levels approximated those of the present day, in doing so populations of **O** became resident in 'higher' areas of the present-day Port Phillip Bay region, where wetter habitats may have prevailed. Subsequent cooling and a lowering of the sea-level may have caused a retraction of lowland species with the coastal habitat; however, it is quite likely that pockets of higher forms may have remained behind. Such a possibility would have given rise to ancestor **R**. As conditions subsequently became moister and warmer, sea-levels are likely to push lowland species and 'highland population isolates' into contact again. Under these circumstances, it is reasonable to assume that the highland populations, providing that sufficient divergence has occurred between them and the lowland ancestor, and having adapted at least in part to their surrounding conditions, would remain in their localized habitat. In theory, a lowland species could either move completely into the highlands to overlap in range, or it could be blocked by either the competitive interactions with a highland species which occupied the relevant aspects of the habitat or a physiological inability to compete successfully. In practice however, it seems that a state somewhere in between these two alternatives is found; I have called this distributional interaction a 'longitudinal sympatry' (see Section 4.3). Longitudinal sympatries can be seen where *E. fultoni* occurs in the highlands, and *E. sericatus* or *E. cunicularius* occurs in the lowlands around the Otway Ranges. It is quite possible that highland species, such as *E. fultoni*, are experiencing a reduction in the distributional range with every rising of the sea-level, and subsequent encroaching of lowland species.

Ancestor **Q**

It is proposed here that prior to the last glaciation, ancestor **Q** (ancestral to *E. quadrimanus* and *E. cunicularius*) was widespread in coastal southern Victoria (which would have included 20-40 m height of sea floor which is currently flooded) in the Bassian Plain and the Gippsland Plain. As the temperatures began to cool and the humidity levels decreased, the

sea-level fell; since the most optimal habitat in terms of moisture would have been close to the coast rather than further inland which would have been drier, the distribution of ancestor **Q** became restricted to (at least) two populations, one in the Bassian Graben, and one in the La Trobe Graben, by the time of prior to the interstadial. The warming (rising sea-levels) effects of the interstadial were probably insufficient to bring these populations together again, and by the time of further sea-level falls the two species had received some degree of differentiation.

The general events which are proposed to have taken place for the *cunicularius* group are depicted in Figure 24.

As stated above the estimation of divergence times is difficult without supplementary information. In a sense the times since divergence are somewhat arbitrary because it is the actual mechanisms which are important in this section, and because sea-levels have fluctuated continuously throughout the late Pleistocene we know that the climatic factors required to produce such a mechanism have indeed occurred. Nevertheless, if we assume that the radiation of *E. cunicularius* has occurred over the last 20 000 years then we can extrapolate to other proposed divergences. Under the assumption that the evolutionary rates along each lineage in this group have been equal, then the larger genetic distance found between *E. fultoni* and *Engaeus* VQ9, compared to that between *E. quadrimanus* and *E. cunicularius*, suggests that the latter two species diverged from each other more recently than the former two species. According to the proposal above, ancestor **O** must have given rise to **R** as the sea-levels rose after the penultimate glacial, approximately 130 000-120 000 y bp.

THE MODEL

Using the *cunicularius* group as an example for the genus *Engaeus*, the following general model can be proposed:

I the plesiomorphic lowland forms are the **'moving front of diversity'**,

II these lowland forms are climatically adhered to the freshwater habitats in the coastal region, following the coast as it rises and falls-**'lowland spread by coastal advance and retraction'**,

III when the sea-levels fall, populations can be left behind by the lowland forms, and these **'highland isolates'** may develop into distinct species provided that they undergo sufficient divergence between the time of isolation and before contact between the lowland and highland forms can be re-established, and these highland isolates develop apomorphic characteristics,

IV if sufficient divergence occurs, and if the highland species and the lowland species come back into contact, then a **'longitudinal sympatry'** between the two species may develop; these sympatries are poorly understood but may effectively block the lowland spread of highland species or vice-versa.

Apart from the possibility of more than two daughter species arising from a single event, as described above, a second implication of this notion is that the species which are not endemic to either Victoria or Tasmania are likely to have been dispersing during the last glacial period. One might, therefore, hypothesize that the entire fauna of a region, such as

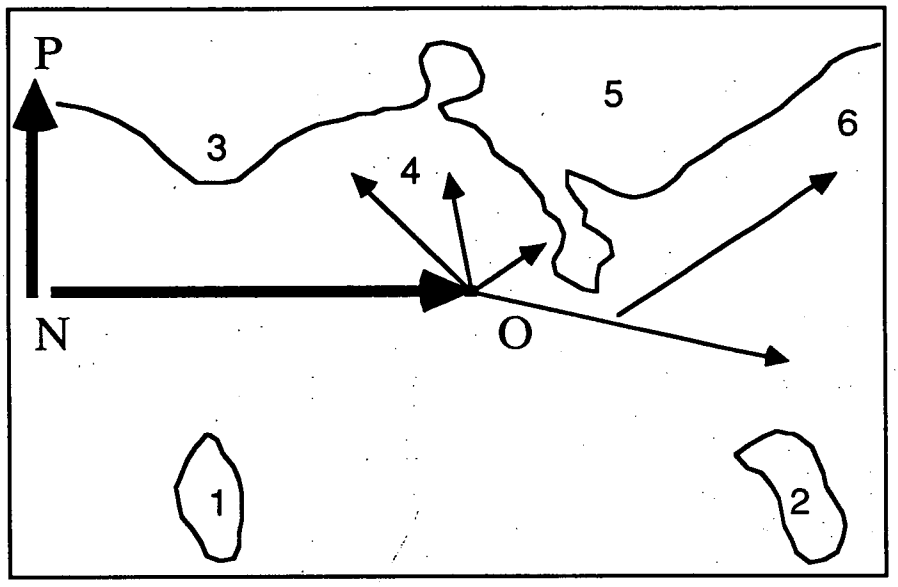
Figure 24: Schematic representation of the speciation events which are hypothesized to have taken place in the *cunicularius* group of species of the genus *Engaeus*, involving ancestral species, showing the approximate position of coastline as it exists today, with King Island (1), Flinders Island (2), Otway Region (3), the central Bassian Graben (4), South Gippsland (5) and the La Trobe Graben (6).

A: The ancestral species **N** splits to form two species **P** (a western lowland form) and **O** (formerly **N**, an eastern lowland form which radiates over south-eastern Victoria).

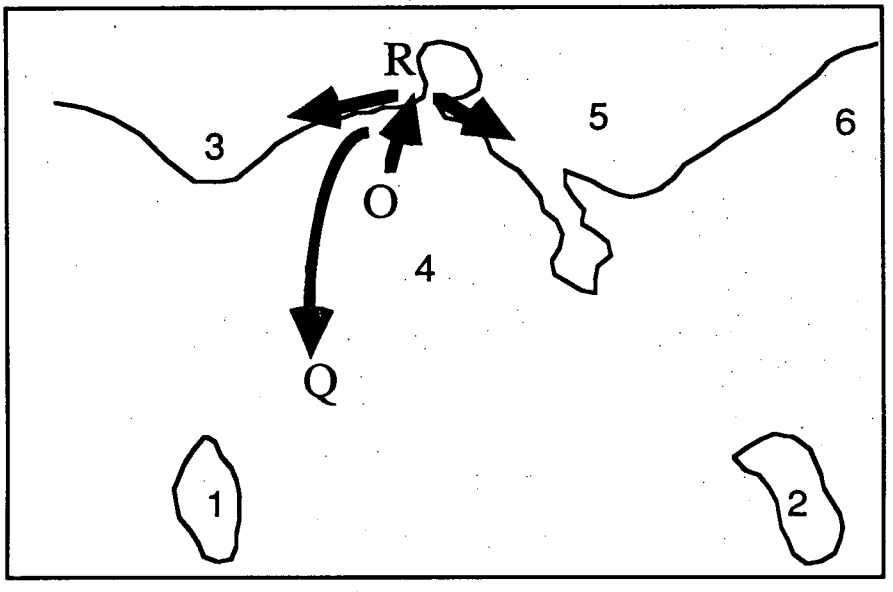
B: seas rise, pushing ancestral species **O** into 'highland' areas; as seas fall again, 'highland' forms become isolated (**R**) (to eventually result in the species *E. fultoni* in the Otway region and *Engaeus* VQ9 in the South Gippsland region) whilst lowland species **Q** -formerly **O**- is retained.

C: Ancestral lowland species **Q** in the Bass Basin and in the Gippsland Basin become isolated (to result in *E. cunicularius* and *E. quadrimanus*).

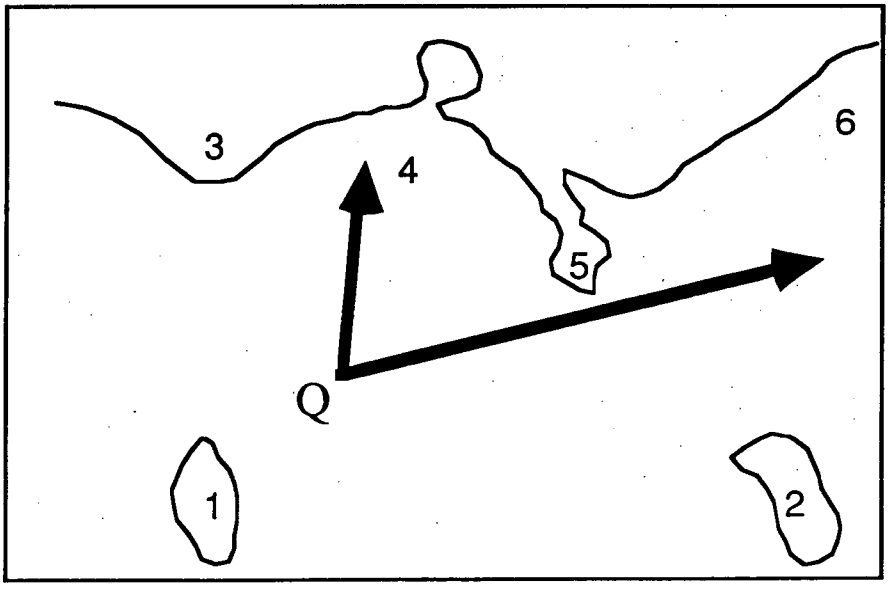
A



B



C



Tasmania, can be examined as a dichotomy, comprising one group where each species is non-endemic and was active during the last glacial period, and another group of endemic species which originated prior to the last glaciation and had therefore developed sufficient divergence from its dispersing ancestor.

OTHER VICTORIAN SPECIES AND SPECIES GROUPS

Ancestor K (Figure 25)

Ancestor **K** is here assumed to be a lowland form, occupying most of coastal southern Victoria. It spread well into the La Trobe River and Yarra River systems, so that when sea-levels dropped and the lowland form contracted with the coast (Ancestor **N**; see above) pockets of populations were isolated in what then became higher regions. In time, the populations may have developed sufficient divergence from **N** to become distinct species (ancestors **M** and **L**).

Ancestor M (Figures 25 and 26)

Ancestor **M** is hypothesized to be an isolated highland species, occupying both components of the Strzelecki Range region, and eventually the western and eastern populations became divergent, resulting in the present day species *E. phyllocercus* and *Engaeus* VRJ respectively. Judging by the amount of genetic and morphological divergence between these two species, such an event may have occurred a considerable amount of time ago.

Ancestor L (Figure 26)

It is proposed here that ancestor **L** was either a lowland or a highland isolate, probably restricted to the upper region of the Yarra River. The mechanism which gave rise to ancestors **S** and **T** is difficult to determine since all the present-day relatives of these ancestors are capable of occupying undulating slopes or highland areas, and because of a complex of parapatric boundaries between at least some of them (a feature that was also noted by Kane, 1964). However, one possible explanation is that ancestor **L** spread into a number of unoccupied habitats, including type 3 burrows in friable gradational soils; if populations in these microhabitats were sufficiently isolated from individuals of the ancestral species **L** the potential for a speciation event might have arisen, to produce ancestor **S**, a highland form occupying type 3 burrows which then migrated north and ancestor **T** (formerly **L**) which inhabited the undulating lowlands of the Upper Yarra River plains. This proposed speciation by microhabitat separation is detailed below.

Ancestor S (Figure 27)

Again, the circumstances which led to the speciation event which ultimately produced the species *E. cymus* and *E. hemicirratulus* is difficult to determine without extra information. However the present-day distributions of these two species suggest that ancestor **S** may have spread widely in highland areas of the Eastern Upland Region during suitable climatic conditions (for instance relatively moist and warm); with any deterioration of these conditions such as extreme cold or drop in the humidity levels (which type 3 burrowers appear to rely upon), the connections between the populations along this range might easily have been broken, effectively isolating them. Perhaps the range was fragmented into two groups, a

Figure 25: Schematic representation of the speciation events which are hypothesized to have taken place for the ancestral species **K**, **L**, **M** and **N**, showing a schematic outline of the Victorian coastline where
1 = Cape Otway,
2 = Wilsons Promontory,
3 = the Bassian Graben,
4 = the La Trobe River and
5 = the Yarra River.
See text for explanations.

Figure 26: Schematic representation of the speciation events which are hypothesized to have taken place for the ancestral species **L**, **M**, **S** and **T**, showing a schematic outline of the Victorian coastline where
1 = Cape Otway,
2 = Wilsons Promontory,
3 = the Bassian Graben and
4 = the La Trobe River.
See text for explanations.

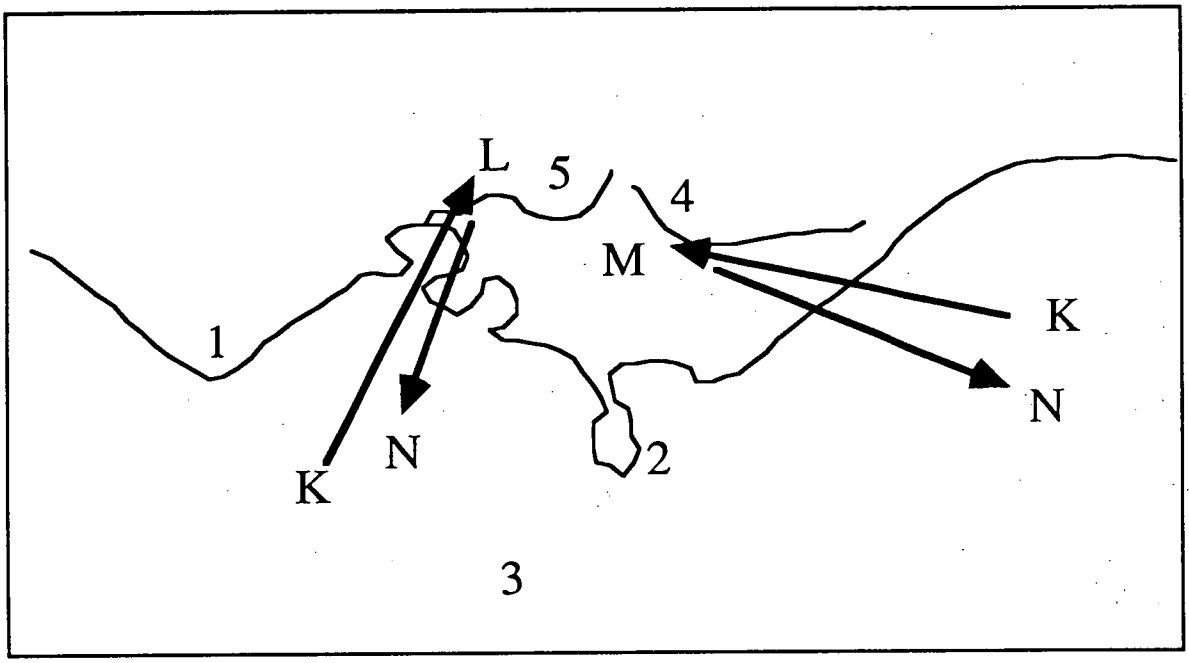


Figure 25

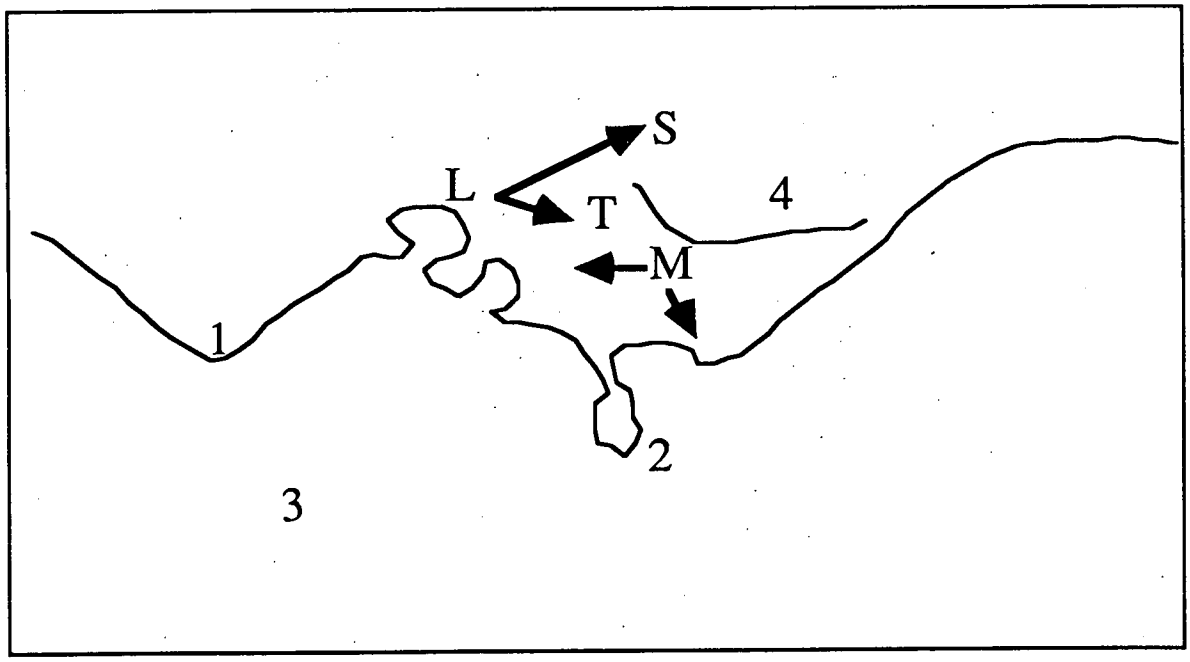


Figure 26

north-eastern group of populations which subsequently adapted to somewhat drier, cooler conditions and evolved into *E. cymus*, and a south-western group which remained wedded to a moist, humid wet sclerophyll habitat with type 3 burrows and is now recognised as *E. hemicirratulus*.

Ancestor T (Figure 27)

Ancestor **T** was probably distributed in all of the western undulating regions of the Eastern Uplands, including the Dandenong Ranges. It is tempting to suggest that the same climatic conditions which saw a fragmentation of ancestor S's range, also contributed to a reduction in the range of ancestor **T**, resulting in, perhaps, ancestor **V** which remained in the Upper Yarra region and ancestor **U** which was geographically isolated on the lower slopes of the Dandenong Ranges.

Ancestor V

Ancestor **V** must have undergone a recent expansion of its range. It would appear that the speciation event which led to the two species of *E. affinis* and *Engaeus* VAFA was relatively recent since the degree of electrophoretic divergence was small (see discussion below). At present *E. affinis* is genetically and ecologically diverse, and a future study of the interpopulational differences for this species might reveal more exacting hypotheses on the speciation of this group as a whole.

Ancestors U and W

Similarly, the genetic distances between the three species which evolved from the ancestor **U** are all relatively small, indicating possible recent speciation events. It is proposed here that the ancestor inhabited the undulating foothills of the Dandenong Ranges; during a period of range or habitat expansion under favorable conditions, the species 'discovered' the type 3 microhabitat which still exists today. The microhabitat separation or specificity may have provided sufficient isolation between the two forms eventually to result in a speciation event which produced *E. victoriensis* and ancestor **W**. A similar proposal for ancestor **W** can be made, but this time the type 3 occupants gave rise to forms which could occupy type 2 burrows in unique flood beds of headwaters of creeks at the top of the Dandenong Ranges, resulting in the two species of *E. tuberculatus* and *E. urostrictus*. The same mechanism can be suggested for *E. affinis* and *Engaeus* VAFA (and indeed was tentatively proposed for ancestors **S** and **T**). In each case where these hypothetical events can be suggested, the extant species concerned

i) show a small amount of both morphological and genetic divergence between the sister species (implying a recent speciation event),

ii) involve restricted distributions where a geographical feature cannot be attributed to a physical barrier between two ancestral conspecific populations,

iii) are capable of occupying different habitats in the same geographical locality, and

iv) exist in sympatry, where the habitat separation is clearly evident (see Horwitz et al., 1985b; see also Habitat Notes for each respective species, Chapter 6).

Figure 27: Schematic representation of the speciation events which are hypothesized to have taken place for the ancestral species **S**, **T**, **U** and **V**, showing a schematic outline of the Victorian coastline where
1 = Cape Otway,
2 = Wilsons Promontory,
3 = the Bassian Graben,
4 = the La Trobe River and
5 = the Yarra River.
See text for explanations.

Figure 28: Schematic representation of the speciation events which are hypothesized to have taken place for the ancestral species **J**, **K** and **Z**, showing a schematic outline of the Victorian coastline where
2 = Wilsons Promontory,
3 = the Bassian Graben,
4 = the La Trobe River,
5 = the Yarra River and
6 = the LaTrobe Graben.
See text for explanations.

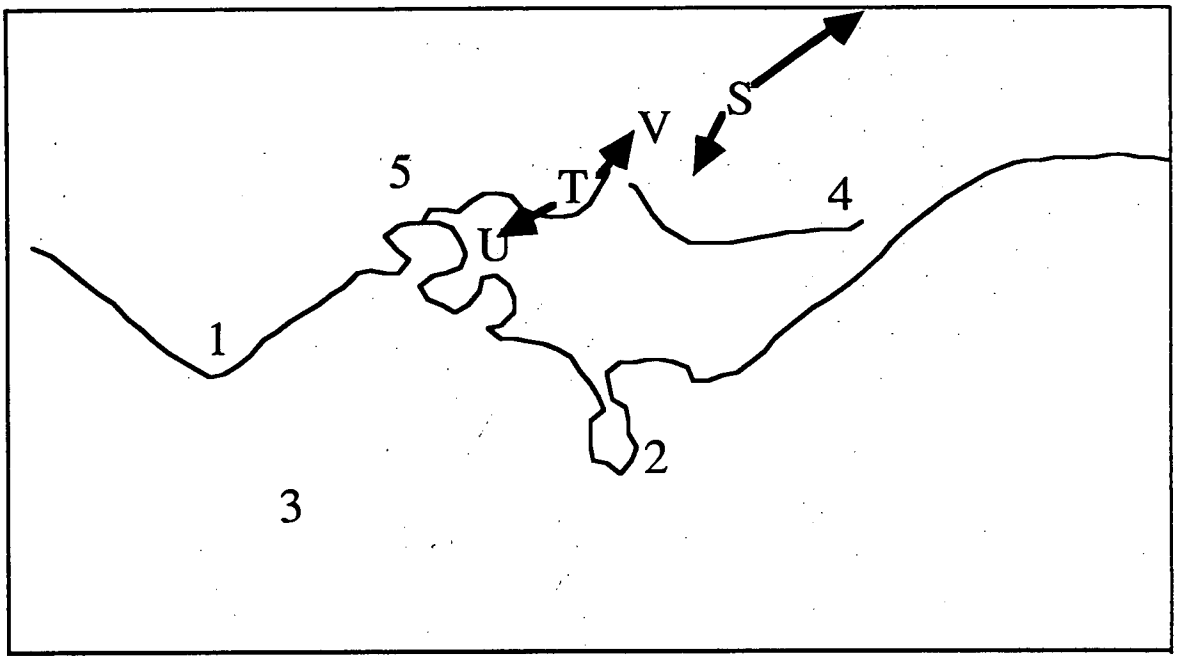


Figure 27

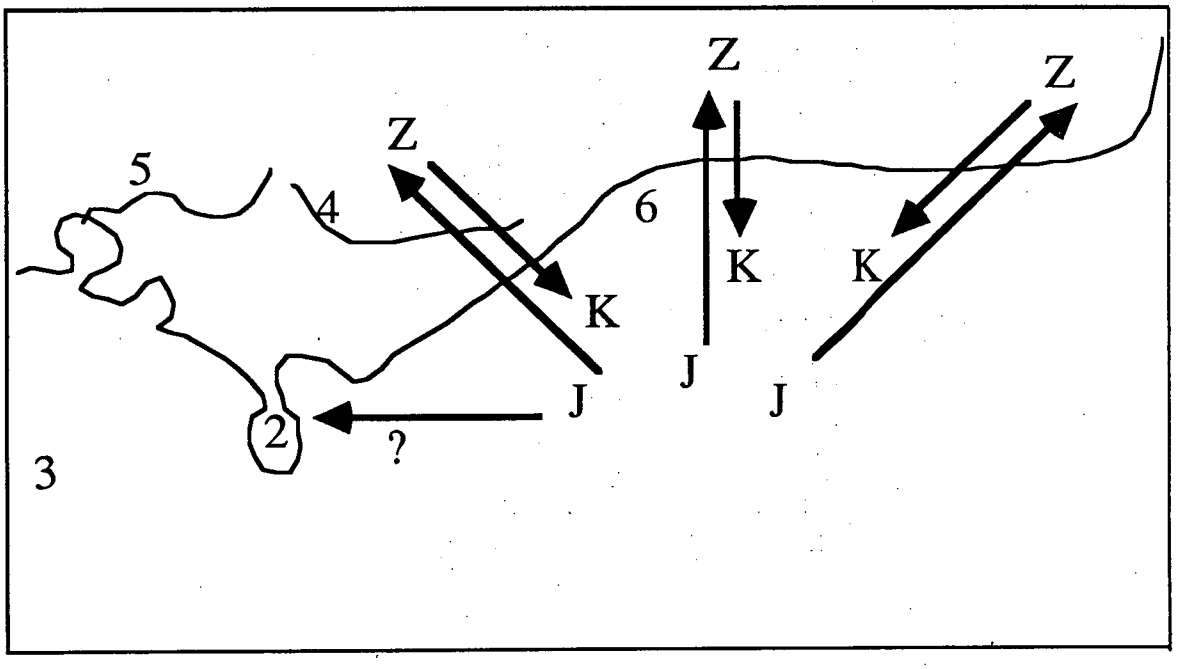


Figure 28

Ancestor J (Figure 28)

An ancestral lowland species **J** is hypothesized to have been widespread in the La Trobe Graben during a period of low sea-levels. When the sea-levels rose, it eventually spread into the undulating hill slopes of the Eastern Uplands, from East Gippsland to the northern slopes of the La Trobe River Valley; with a subsequent retraction of the sea-levels, populations were left in this region, whilst again, a lowland form followed the coast to occupy its former position in the La Trobe Graben (Ancestor **K**). The remaining populations, diverging to evolve into ancestor **Z**, might well have been in panmixia across their range but eventually it is hypothesized that a clinal divergence occurred to produce the species *Engaeus* VSL in far eastern Victoria (such a clinal divergence might be exactly the same process which *E. quadrimanus* is undergoing now in the same geographical area, where its far eastern populations appear to be losing their pores and spines on the tail fan). The stock might then easily be divided into two, perhaps with a central aridity as is present now being the major cause. The results of such a division would be *E. orientalis* in the east and *E. sternalis* in the west.

The enigmatic *E. australis* might have been 'deposited' on Wilsons Promontory during this particular fluctuation in sea-level.

TASMANIAN SPECIES

Ancestor E (Figure 29)

This species is hypothesized to have occupied coastal habitats in the Tamar Basin; probably as a result of a rise in the sea-levels, with their associated change in vegetation and climate, and resultant drop in sea-level with a cooling of temperatures, left an 'highland' ancestor **X** and the contracting remnant populations belonging to ancestor **F** (formerly **E**). In addition ancestor **E** probably gave rise to the ancestor of *E. fossor* in the north-west of the Tasmania.

Ancestor X (Figure 29)

Ancestor **X** is likely to have been widespread in the north-east of Tasmania and on the Furneaux Group of islands. The most likely course of events is for this species to become gradually fragmented, firstly by division of its habitat resulting in *Engaeus* TA. This species appears to have subsequently suffered a severe reduction of its range, and this may be due to an aridity in the north-east of Tasmania at least during the last glacial period, (as evidenced by sand dune systems, Bowden, 1981), during which *Engaeus* TA was restricted to relictual populations where suitable conditions prevailed. *E. leptorhynchus* and *Engaeus* TQ appear to have become isolated from each other by successive floodings between the north-east of Tasmania and the Furneaux Group. Since their respective isolation, *Engaeus* TQ appears to have reduced its range although its absence on Cape Barren Island has yet to be established. *E. leptorhynchus* may have recently expanded its distributional range since it now occupies some habitats of over 700 m (for instance at Blue Tier) where alpine conditions would have prevailed during the last glacial period (22 000 y bp; Kirkpatrick, 1986).

Ancestor F

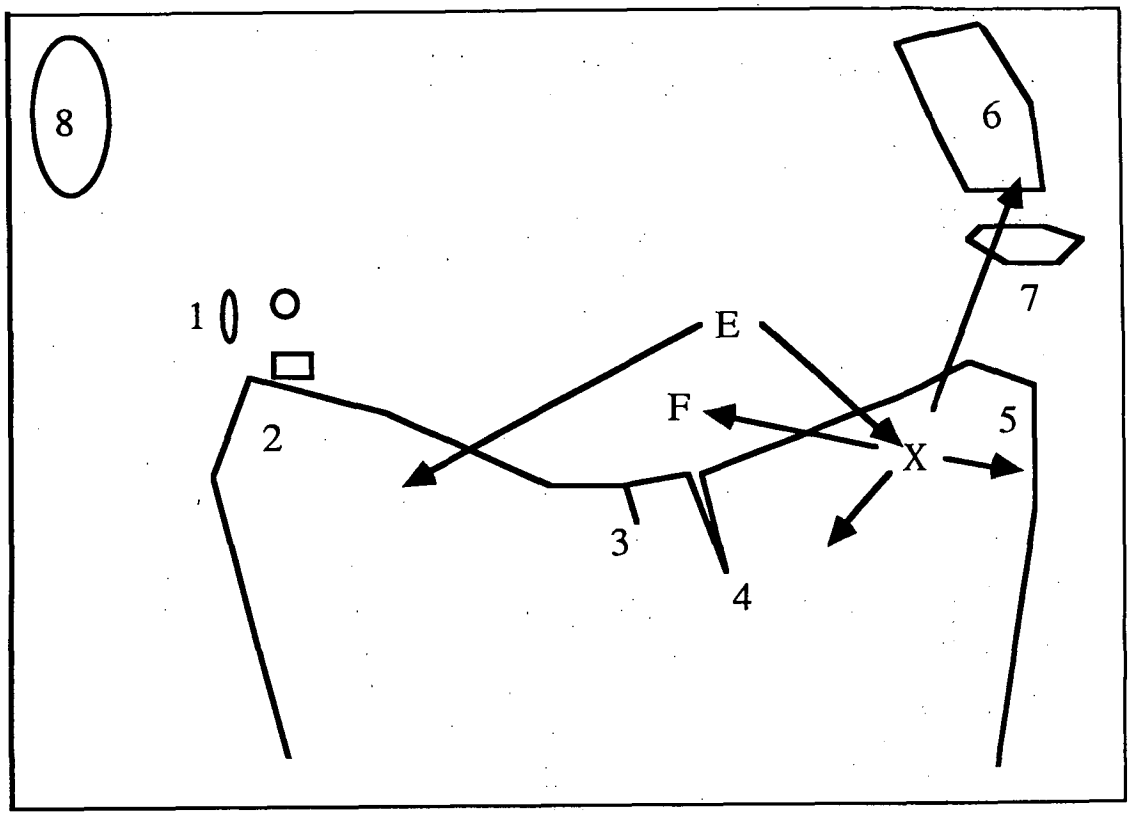


Figure 29: Schematic representation of the speciation events which are hypothesized to have taken place for the ancestral species **E**, **X** and **F**, showing a schematic outline of the Tasmanian coastline where

1 = Hunter Island,

2 = north-western Tasmania,

3 = Port Sorell,

4 = Tamar River,

5 = north-eastern Tasmania,

6 = Flinders Island,

7 = Cape Barren Island and

8 = King island.

See text for explanations.

The ancestors to the remaining members of the *fossor* group are represented diagrammatically in Figure 30; ancestor **F** underwent a speciation event to produce ancestors **G** and **H**. The former species eventually gave rise to *Engaeus* TF, a species which may well have undergone significant reduction in its range (for the same reasons as described for *Engaeus* TA). This may be due to a possible habitat specificity for buttongrass plains. Ancestor **H** became widespread in the lowland region of Tasmania and it is hypothesized here that a group of populations became resident in the Tamar Graben behind what is now Launceston, whilst the remaining populations of **H** retreated to the coastal habitats as the sea-levels decreased (to become ancestor **I**). With an increase in sea-levels associated with the most recent glacial, ancestor **I** retracted, and formed two groups of populations, one smaller group on the western side of the Dazzler-Asbestos Range region, one group on the eastern side; this region may well have been sufficient to isolate these two populations to result in the species *Engaeus* TB and *Engaeus* TBZ.

Ancestor **A** (Figure 31)

Ancestor **A** existed throughout the Bassian region, in the Bass, La Trobe and Western Grabens. Despite the unresolved and speculative nature of the phylogeny presented in Figure 22, it can be hypothesized that ancestor **A** gave rise to a multiple fragmentation event as sea-levels rose and subsequently fell again, to leave several isolates. In Tasmania, ancestor **A** moved into the Tamar River region with the rising of sea-levels, and with their subsequent lowering, ancestor **C** was left as a 'highland remnant' and ancestor **E** retreated with the coast to remain as a lowland species. Along the western side of the Bassian Rise ancestor **B** became isolated from its lowland ancestor, and finally, in the lowland regions of Victoria, ancestor **A** gave rise to ancestor **J**.

Ancestor **B** (Figure 31)

This species became widespread along the western portion of the Bassian Rise. Eventually the Tasmanian and Victorian populations became geographically, and eventually reproductively isolated, through the effect of rising waters and gradual geographical separation of populations, with *E. strictifrons* spreading into western Victoria and *Engaeus* TJ dispersing in western Tasmania. The absence of a remnant of ancestor **B** on King Island may be the result of a reduction in suitable habitat followed by a period during which remnant populations were unable to recolonize areas producing a gradual extinction.

Ancestor **C** (Figure 31)

This species became distributed along the region now corresponding to the north coast of Tasmania, where presumably it occupied wetter, higher areas. However with a rising of sea-levels it is quite possible that north-eastern and north-western populations could have been isolated by the Tamar Graben, and if this were so, then eventually two isolated species would evolve, namely *Engaeus* TD in the north-east and an ancestor of *E. cisternarius* and *Engaeus* TN in western and central regions. Judging by the low levels of genetic and morphological diversity between the two latter species, it is proposed here that they separated relatively recently.

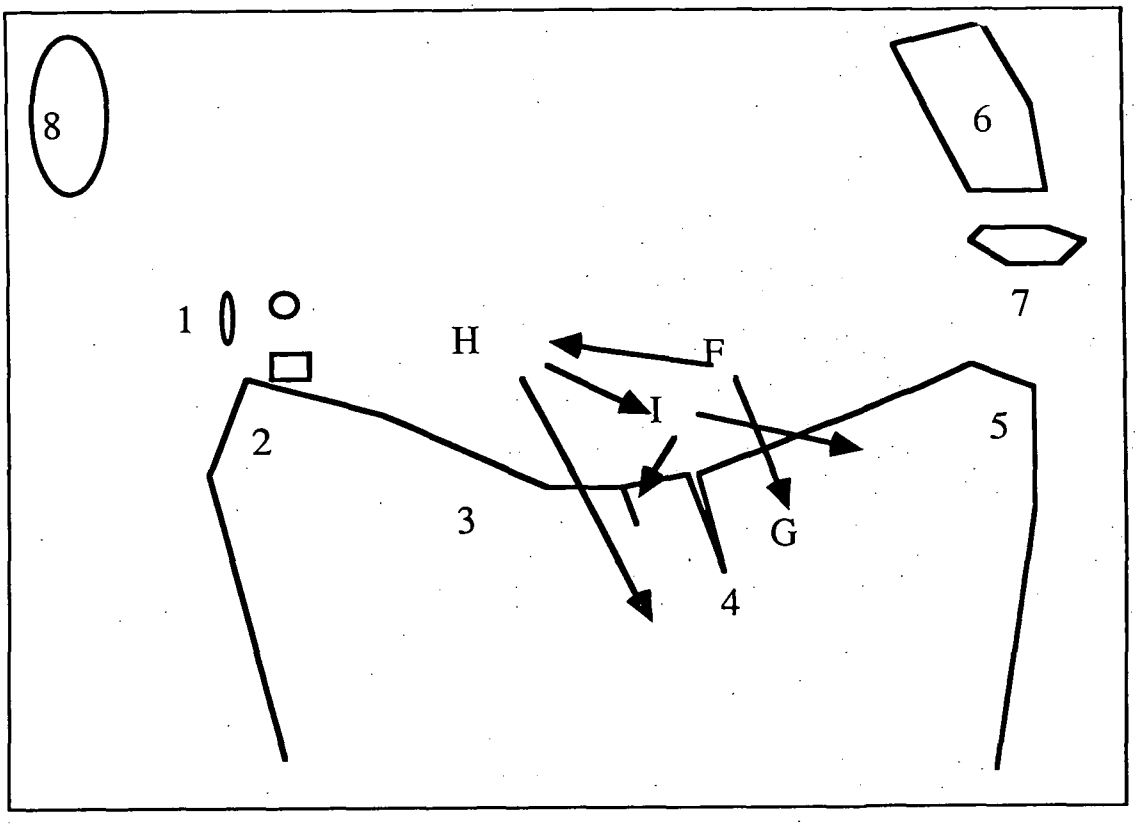


Figure 30: Schematic representation of the speciation events which are hypothesized to have taken place for the ancestral species **F**, **G**, **H** and **I**, showing a schematic outline of the Tasmanian coastline where

1 = Hunter Island,
2 = north-western Tasmania,
3 = Port Sorell,
4 = Tamar River,
5 = north-eastern Tasmania,
6 = Flinders Island,
7 = Cape Barren Island and
8 = King Island.

See text for explanations.

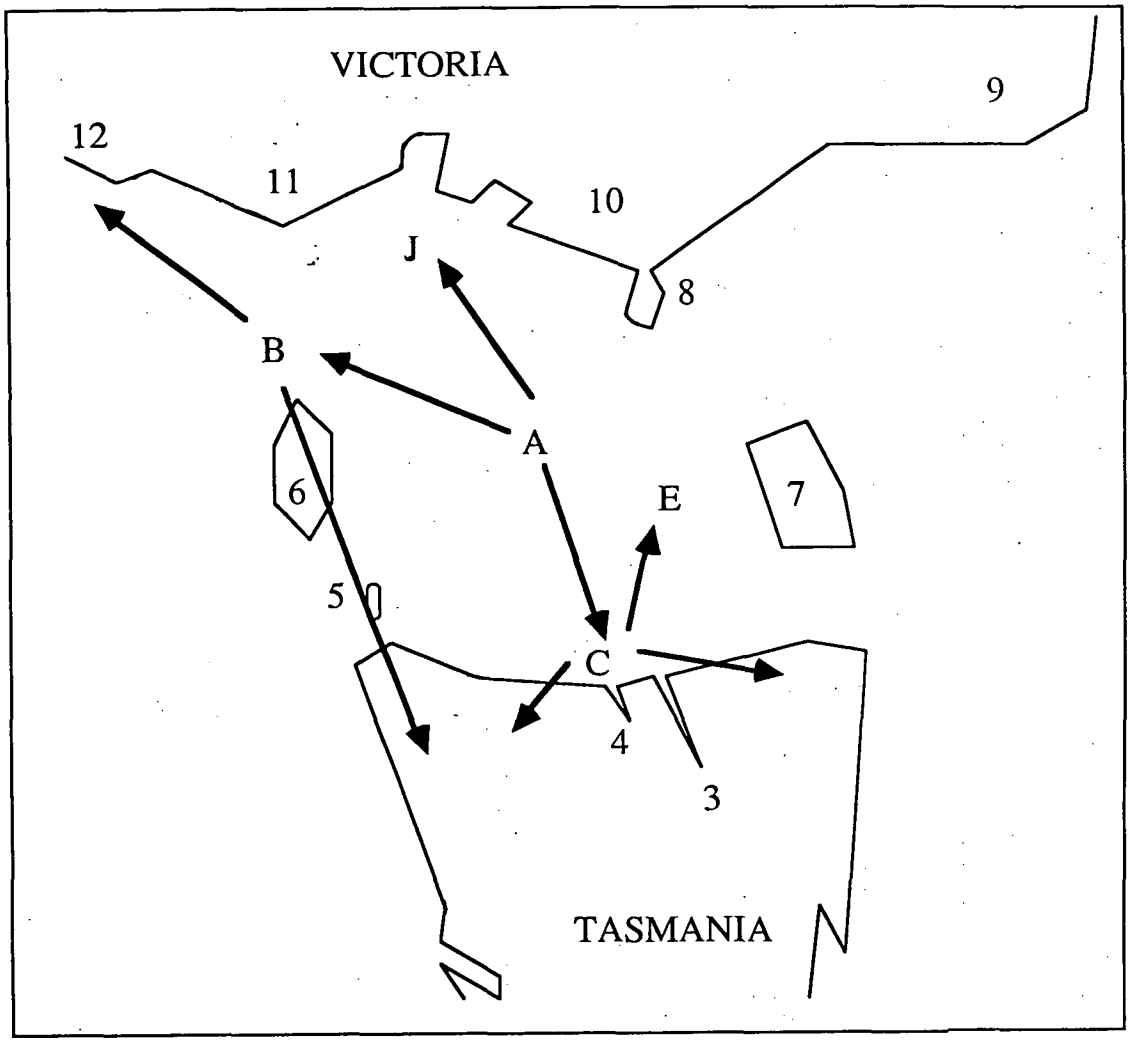


Figure 31: Schematic representation of the speciation events which are hypothesized to have taken place for the ancestral species **A**, **B**, **C**, **E** and **J**, showing a schematic outline of the Tasmanian and Victorian coastline where
3 = Tamar River,
4 = Port Sorell,
5 = Hunter Island,
6 = King Island,
7 = Flinders Island,
9 = East Gippsland,
10 = South Gippsland,
11 = Otway Ranges and
12 = western Victoria.
See text for explanations.

OTHER SPECIES, GENERA

Whilst the positions of *E. laevis*, *E. lyelli*, *Gramastacus*, *Engaewa*, *Geocharax* and *Tenuibranchiurus* in the proposed phylogeny must be considered as highly speculative, certain aspects of the historical factors which have led to their present-day distributions, can be discussed.

Engaewa spp.

The results of an electrophoretic analysis of this genus, using members of the genera *Engaeus* and *Tenuibranchiurus* as outgroups, suggests that all of the species of *Engaewa* in Western Australia are more closely related to each other than they are to other species of parastacids (Horwitz, unpublished data). This information suggests that the present-day distributions of this genus are the results of one ancestral dispersal event, followed by more recent speciations in the south-west of Western Australia. Assuming that the origins of this genus are from the Bass Strait region, there are two possible routes which such an ancestor may have taken. It may have dispersed across what is now known as the Nullarbor Plain during the wetter and warmer conditions of the Tertiary, where rainforest provided a 'corridor' from east to west. Such a route would however, have been unlikely since present species of this genus occupy burrows in heath vegetation in sandy coastal plains. Perhaps more plausible is the hypothesis that the ancestor of *Engaewa* dispersed during times of lower sea-levels, when a broad stretch of sandy coastal plains would have bordered the current position of the continent, and at one stage probably approximated the conditions in which species of this genus can now be found. This hypothesis is supported by the assertions of Galloway and Kemp (1981; cited in Section 4.6), on the opportunities for the dispersal of psammophilous biota during the Pleistocene.

Geocharax sp.

The mechanism of 'lowland spread by coastal advance and retraction' can be used to explain the occurrence of both *Geocharax* sp. and *Engaeus laevis* in Victoria and Tasmania, however it is proposed here that these two species did not originate in the Bass Strait basin itself, but rather on either side of it. *Geocharax* sp. probably was found west of the Cape Otway-King Island-northern Tasmania link when the sea-levels were at their lowest; as they rose and separated King Island and Cape Otway, populations west of the Otway Ranges and south-west of King Island then became isolated, and further rising resulted in the isolation of populations on King Island and north-west Tasmania. This proposal may suggest a reason why *Geocharax*, a relatively vagile species of crayfish, does not occur in north-eastern Tasmania or along the eastern side of Bass Strait. However the occurrence of a population of *Geocharax* at the base of the Western Strzelecki Range is somewhat anomalous.

Engaeus laevis

E. laevis was probably found in the La Trobe Graben at the time of lowered sea-levels, and as the levels rose it gradually expanded its range. Presumably this expansion at the height of the sea-levels included populations in north-east Tasmania, the Furneaux Group and the Gippsland Plains. The species may have been subsequently lost from Flinders Island

and other Bass Strait islands due to a bottleneck effect (assuming of course that sampling has not failed to locate the species). The origins of *E. laevis* have been put at this multiple fragmentation but its position is certainly in doubt since it can not be satisfactorily incorporated into the phylogeny.

Gramastacus, E. lyelli and *Tenuibranchiurus*

The absence of all of these species from Tasmania and the Bass Strait Islands suggests that they have been absent from the Bass Strait region for a considerable period of time. It is quite likely then that an ancestor of *Gramastacus* and *E. lyelli* spread from south of the Great Divide, probably from the West Victoria Plains Region, to just north of the Great Divide and to the Grampian Range region. Perhaps at the same time the ancestor spread in a eastward direction along the coastal margin, where the species ramified northwards to eventually reach its apparent northern extremity in coastal Queensland (*Tenuibranchiurus*). If this above hypothesis were to be correct, then one might expect to find pockets or remnants along this route; the occurrence of a new species with *Gramastacus*-like features near Wyong and Forster in coastal New South Wales concurs with this expectation.

SUMMARY

According to the above hypotheses, there are four major models of speciation for the species in the genus *Engaeus*, namely

i) isolation by fluctuating sea-levels,

ii) isolation by fragmentation of a range,

iii) clinal divergence at the periphery of a range, and

iv) isolation by microhabitat separation.

These are discussed further in the next section.

Based on the levels of endemism in both Victoria and Tasmania, the Bass Strait appears to have been a major obstacle to dispersing crayfish, and this is despite the fact that present sea-levels in Bass Strait are considered to be higher than average, thus portions of the Bassian Rise would have been exposed for quite large periods of time. However, according to the discussion above, there appear to be two major exceptions to this generality. The first is for the most recent glaciation, since it is hypothesized that during this period, three species were able to disperse to Victoria, Tasmania and perhaps islands in the Bass Strait from lowland basins (now submerged). The second exception is more speculative because it must have occurred a considerable period of time ago, during a glaciation which provided the impetus for the major split between the Victorian and Tasmanian crayfish faunas. Otherwise, all speciations in Victoria and Tasmania appear to have occurred locally without the isolating effect of Bass Strait.

Section 4.8 DISCUSSION

Endemism

It has been demonstrated that the crayfish species, belonging to the five closely related genera which have been discussed in this chapter, are largely restricted to the Bassian biogeographical region. In fact only *Tenuibranchiurus* is found outside the Bassian region. Of the remaining four genera, each has showed a distributional restriction to a particular area within this region. *Engaewa* is found only in the south-west of Western Australia, *Geocharax* is found predominantly in Victoria and Tasmania and *Gramastacus* is found predominantly in Victoria. *Engaeus*, the most diverse genus, exhibits a very high level of regional endemicity in both Victoria and in Tasmania, and within each State, the endemism within selected physiographic regions can also be very high.

A high level of endemicity for the Tasmanian fauna was proposed by Bayly and Williams (1965) and has since been described for species of selected invertebrate groups, including the megadrile Oligochaeta (Jamieson, 1974), the blephariceridid Diptera (Zwick, 1977), Trichoptera (Neboiss, 1977), Plecoptera (Hynes and Hynes, 1980), terrestrial amphipods (Friend, 1980) and psephenid Coleoptera (Davis, 1982). In addition, Williams (1974b) suggested that the Tasmanian freshwater Crustacea are noted for their high level of both diversity and endemicity; this suggestion appears to have been substantiated by the work of Knott (1975) and by the information presented in this study.

Friend (1980) compiled a list of the better-known groups of non-marine fauna which occur in both Tasmania and Victoria, including those above, and demonstrated a close correlation between endemicity in Tasmania and levels of vagility. This information is thus in accordance with the results presented herein, where high levels of endemicity in Tasmania seem to be correlated with relatively low levels of vagility. In fact the two non-endemic species of *Engaeus* in Tasmania exhibit a greater capacity for mobility than the majority of the remaining species.

It has been suggested (for example by Friend, 1980; Hynes and Hynes, 1980) that the conditions in Bass Strait during the last glaciation would have been largely unsuitable for the dispersal of organisms, in particular freshwater ones. Dunes developed on the exposed floor of the Bass Strait (and in north-eastern Tasmania) during the last glaciation and the resulting sandy surface would have restricted migration of many species while major contrasts between the environments of Tasmania and Victoria would also have helped to preserve the differences between their biota. However, if swamps in coastal habitats in at least isolated areas remained moist for a large part of the year, then animals with an ability to survive short periods of partial dessication, for instance burrowing species such as those in the genus *Engaeus*, might well have had the opportunity to disperse across Bass Strait according to the mechanism described in the previous section.

Therefore, it appears that in general the Bass Strait was an effective barrier to the dispersal of some organisms, particularly those with a lower vagility, but that the opportunity for some selected species under certain circumstances to migrate north-south or south-north

may have existed at some stage during glacial periods when a land connection was present. Viewed under these restraints it has not been surprising to find a minority of recently dispersed species of the genus *Engaeus* in Tasmania.

The high levels of endemism within Victoria appears to be attributable to the interaction between sea-level fluctuations and ecological factors. For instance it was proposed that an increase in the habitat diversity provided by a topographical complexity contributed to the high levels of both regional diversity and endemicity in the South Victoria Uplands and to a lesser extent the East Victoria Uplands and including the 'islands' of Wilsons Promontory and the Furneaux Group which each contain one endemic highland species. Conversely, regions of low topographic variability exhibited low levels of both diversity and endemicity, for instance the West Victoria Plains. Similar results have been obtained independently by Abele and Blum (1977) and Chace and Hobbs (1969) who examined the freshwater decapod fauna of two different groups of Carribean islands, and recorded the same correlation between number of species and elevation. In addition Abele (1974), although working on marine decapods, demonstrated that the number of species in a habitat was a function of the structural complexity of that habitat. Such references suggest that the lowland-highland hypothesis proposed here has the support of decapod studies in other areas of the world.

The situation in Tasmania is less clear than that found for Victoria. Here the physiographic regions are more difficult to delineate. In the north-west of the state it was proposed that the comparatively homogeneous climate and vegetation patterns provided the opportunities for the widespread distributions of the three species of *Engaeus* which are endemic to this particular region (*fossor*, *cisternarius*, and TJ). Compared to the north-west of Tasmania, the north-east displays a more varied rainfall pattern and this may be associated with a varied vegetation pattern (see Kirkpatrick and Dickinson, 1984). In addition the past climatic events in the north-east of the State culminating, for instance, in alpine conditions over large areas (Kirkpatrick, 1986) and sand dunes around the Bridport area (Bowden, 1981), might have been influential in confining species to relictual areas of suitable habitat; this effect may still be in evidence for the species *Engaeus* TA and *Engaeus* TF. Thus the forces acting in the north-east of Tasmania may have been more akin to the 'refuge' hypotheses proposed for other areas in the world.

To my knowledge, no other diverse group of species has been examined for areas of comparative endemicity in the Bassian region. This is unfortunate since it largely prevents the 'testing' of the notions presented above. Nevertheless this work will perhaps allow such a test in the future.

One group of organisms which shows perhaps the greatest potential in this regard is the freshwater crayfish group of *Astacopsis* and *Euastacus*. These genera apparently come from the same ancestral stock, which differs to that monophyletic group investigated in this study, according to the phylogenetic interpretations of Figure 22. The latter genus has been shown to be diverse in Victoria, particularly in eastern and southern areas in highland localities (Morgan, 1983) although distribution maps are not yet available. *Astacopsis* is

currently recognised as two species with well defined distributional ranges in Tasmania (Swain et al., 1982); however recent evidence based on the shape of the sternum has suggested that this genus may be more diverse than this (Horwitz, unpublished observations). Judging by their external morphology, *Astacopsis* and *Euastacus* are clearly closely related (if not the same genus). In fact they were shown to be virtually indistinguishable immunologically (Patak and Baldwin, 1984). The potential exists, therefore, for a comparable study to be undertaken on this group.

Speciation

The major model of speciation proposed in the preceding section, that of 'lowland spread by coastal retraction - highland isolation' probably corresponds most closely to the "Model II", or the peripheral isolates model of allopatric speciation proposed by Wiley (1981) where a multiple furcation or polytomous event can occur with each 'highland isolate' being small in size and more apomorphic than the central population.

It is important to note that this method of dispersal across the Bass Strait, resulting in a speciation event, differs from the usual mode discussed in the literature, namely that of traversing land bridges (see for example Rawlinson, 1974). Williams' (1974b) notion that Tasmanian species of *Engaeus* and *Geocharax* are the results of '...localized isolation of fragments of ancestral populations which inhabited southern Victoria, northern Tasmania and an exposed area between now submerged by Bass Strait...' conforms in part to the hypothesis provided in this thesis.

The discussion of amphibian dispersal and subsequent speciation is probably the most relevant to this hypothesis. For instance Littlejohn (1967) proposed an expansion of amphibian fauna during glacial periods, particularly in a westerly direction. With the interglacial conditions the sea level rose and the southern Bassian fauna withdrew into higher elevations and assumed its fragmented pattern (Littlejohn, 1967). Moore (1954) and Keast (1961) hypothesize a 'double migration pattern' from Victoria to Tasmania during successive isolations of the Bass Strait, for frogs and birds respectively. Littlejohn (1967) on the other hand proposed an initial migration from Victoria to Tasmania during glacials, differentiation whilst in isolation and subsequent reinvasion to Victoria by the differentiated species, to account for frog speciation in the Bassian Region.

A second speciation mechanism proposed in this thesis is for a widely distributed ancestral species to become fragmented by an extrinsic barrier into two groups of populations which subsequently diverge to become reproductively isolated. This mechanism might act, for instance, where climatic conditions produce a reduction in rainfall and fragment one continuous population into two discontinuous ones. This common mode of speciation, one of 'large scale geographic disjunction', corresponds to the "Model I" of allopatric speciation of Wiley (1981), to the "Type Ia" of allopatric speciation of Bush (1975) and to the "adaptive divergence" model of Templeton (1981).

Speciation events arising from clinal divergence (sensu Templeton, 1981) have been proposed for one species, *Engaeus* VSL. Intraspecific clinal variation has indeed been

detected, for instance electrophoretically for *E. hemicirratulus* (see Chapter 2) and morphologically for *E. quadrimanus* (see Species Description, Chapter 6). However, without additional information it is very difficult to predict whether clinal variation will result in a speciation event. Similarly the results of an ancestral clinal divergence will be difficult to distinguish from the other forms of allopatric speciation as discussed above. Whilst recognizing the possibility of the occurrence of such mechanisms, it is for the above reasons that clinal divergence has not been proposed as a major speciation mechanism in this thesis. It has been proposed here due to the occurrence of the same clinal variation in important taxonomic, morphological characters for a lowland species (*E. quadrimanus*) in exactly the same geographical region. Even so this evidence is by no means conclusive and needs to be investigated further.

Finally, the microhabitat separation model of speciation for this genus is also speculative. It originates from the detection of species existing in sympatry which are their closest living relatives and are furthermore not greatly divergent. It is based upon the assumption that a microhabitat separation, usually between type 2 and type 3 burrow habitats, is sufficient to result in a gradual reproductive isolation between the populations occupying each microhabitat. In attempting to fit this model to those proposed for species in general, the situation becomes somewhat confused, mainly, it seems, due to terminology. Most authors agree that such a habitat segregation can indeed lead to a speciation (Wiley, 1981). Some authors are of the opinion that such speciation is merely 'allopatric' since populations are effectively 'geographically separated' (Mayr, 1963). Proponents of the existence of parapatric or sympatric speciation believe that such a speciation can occur within the natural range of movement of individuals of both populations, but that the actual process of speciation depends upon the order in which premating reproductive isolation and the shift to a new niche occurs (Bush, 1975).

Thus, any further work on this particular mechanism should include in its agenda an examination of the possible modes of premating isolation.

Biogeographical Hypotheses

The ideas presented above, namely the proposed speciation mechanisms and each individual speciation event, are effectively biogeographical hypotheses which need to be tested. Obviously, the simplest way to falsify the hypotheses is to provide evidence for the falsification of the phylogenetic hypotheses, upon which the zoogeography is based. Additionally, the interpretations of the climatic changes can be falsified by the presentation of new and contradictory information. Consequently there exists much scope for further work in testing each of the individual hypotheses presented in this chapter.

Owing to the largely speculative nature of zoogeographical studies, suitable techniques for testing hypotheses as a whole have been fairly thin on the ground. Recently, however, the work of Rosen (1978) and Platnick and Nelson (1978) has provided zoogeographers with a technique to analyse regional historical biogeography. They suggest that by constructing a cladogram based on vicariant events, they can predict that, because

unrelated taxa experience a common history of vicariant events, there should be concordance in their estimated phylogenies. This technique can then be used to 'test' zoogeographical hypotheses.

Objections to the use of concordance cladograms for testing zoogeographical hypotheses have been raised by Endler (1982), based upon an inherent level of error in estimating vicariance patterns.

Other problems with this approach include finding another group of species (apart from the one under investigation) which can be easily interpreted, has good distributional data, and has phylogenetic hypotheses already constructed for it. In addition the group must have a compatible overall geographical range. (The geographical range aspect of this technique appears to be quite limiting, particularly for the examination of hypotheses relating to diverse faunas in a relatively small geographical area, as is the case for the genus *Engaeus*. In fact the technique has prevalence in the examination of inter-continental differences.) The approach outlined above is relatively new to the science of biogeography, consequently the chances of finding such a study are slim, and in fact a congruent study of a diverse monophyletic group occurring in both Tasmania and Victoria has not been undertaken prior to this one. Consequently this approach can not be pursued here. However its presentation allows for further researchers to compare distributional patterns and perhaps even construct concordant cladograms. In particular, the proposed study for the *Astacopsis-Euastacus* group should be treated in this way.